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TERMIS-EU Meeting Abstracts

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(1) 3D Co-culture of Human Endothelial Cells and Myofibroblasts

Pullens R.A.A., Stekelenburg M., Bouten C.V.C., Baaijens F.P.T., Post M.J.

Eindhoven University of Technology, Faculty of Biomedical Engineering, P.O. Box 513, 5600 MB Eindhoven, The Netherlands

Introduction: Endothelial cells (ECs) are important for vascular tissue engineering. Seeding of these cells on cultured grafts is often done 2–3 days prior to implantation. Although in several animal models a confluent endothelium was found, it cannot be excluded that this was caused by host derived re-endothelialization. In humans re-endothelialization of grafts is slow and almost never complete. A functional endothelial layer should, therefore, be created *in vitro*. This might require a longer culture period of ECs, which could influence the tissue development. The goal of this study was to increase the EC culture time on engineered vascular constructs, while retaining the structural tissue properties.

Methods: Rectangular PGA/P4HB scaffolds were seeded with human saphenous vein myofibroblasts using fibrin. The constructs were constrained and statically cultured for 5 weeks. ECs were cultured on the constructs for the last 1 or 2 weeks, using EC medium. Afterwards, the confluency of the EC layer was determined as well as the ECM composition and mechanical properties of the constructs.

Results: After 1 and 2 weeks, a confluent monolayer of ECs was found. All constructs possessed abundant amounts of collagen, resulting in strong tissues. The tissue composition and mechanical properties of the EC seeded constructs were comparable to the non-EC-seeded controls.

Conclusion: By changing the culture medium, a confluent endothelial layer was created on strong cardiovascular constructs. In the future, this method will be applied to tubular constructs in a bio-reactor to create small diameter vascular grafts.

(2) A Composite Scaffold for the Engineering of Hollow Organs and Tissues

Eberli D., Yoo J.J., Atala A.

Institute for Regenerative Medicine, Wake Forest University School of Medicine and University of Zurich, Department of Urology, Switzerland

Introduction: Several types of synthetic and naturally derived biomaterials have been used for augmenting hollow organs and tissues. However, each has desirable traits which were exclusive of

the other. We fabricated a composite scaffold and tested its potential for the engineering of hollow organs in a bladder tissue model.

Materials and methods: The composite scaffolds were configured to accommodate a large number of cells on one side and were designed to serve as a barrier on the other side. The scaffolds were fabricated by bonding a collagen matrix to PGA polymers with threaded collagen fiber stitches. Bladder cells were seeded on the composite scaffolds, and implanted in mice for up to 4 weeks and analyzed.

Results and discussion: The composite scaffolds, consisting of an acellular matrix and PGA polymers, are tightly bonded, and maintained their structural properties. The bladder cells readily attached and proliferated on the scaffolds and formed bladder tissue structures *in vivo*. The biomechanical studies demonstrated that the tissues were readily elastic while maintaining their pre-configured structures.

Conclusion: We demonstrate that a composite scaffold can be fabricated with two completely different polymer systems for the engineering of hollow organs. The composite scaffolds are biocompatible, possess ideal physical and structural characteristics, and are able to form tissues *in vivo*.

(3) A Cross-Linked Collagen Scaffold as a Vehicle for Gene Delivery

Viñas Castells R., Breen A., Damodaran G., Ritter T., O'Brien T., Pandit A.

National Centre for Biomedical Engineering and Science, Regenerative Medicine Institute, National University of Ireland, Galway, Ireland

Successful gene therapy is hindered by a short duration of transgene expression (1). Continuous therapeutic gene delivery through scaffolds is therefore needed for treatments of localised diseases. It is hypothesised that a cross-linked collagen scaffold will provide a system for prolonged gene expression.

Collagen gels (3 mg/ml) were fabricated and cross-linked using 40% (v/v) ethanol solution containing 50 mM 2-morpholinoethane sulfonic acid, 5 mM of N-hydroxysuccinimide and three different 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide (EDC) concentrations. Scaffolds were cross-linked overnight, and freeze-dried. A set weight of each sample ($n=3$) was incubated in collagenase solution. Reaction stopped at varying time-points and remaining sample weight recorded. Adenovirus encoding GFP (5×10^7 pfu/ml) in 500 μ l DPBS was placed on scaffolds and supernatant collected at particular time-points. Sponges were degraded overnight

in collagenase solution. An ELISA was carried out to detect viral particles in supernatant and degraded sponge solution using detection antibody adenovirus type 5. Human fibroblasts (HFFF) were transfected with 20% viral supernatant and degraded sponge solution to assess bioactivity of virus.

Degradation assay showed the 40 mM EDC cross-linked scaffolds degraded at a constant rate. ELISA showed that the 40 mM EDC absorbs viral particles at a constant rate (peak at 24 hours). HFFF were transfected with virus retained in scaffolds, seen by GFP expression. It is concluded that a vector release rate proportional to the degradation rate of the vehicle while maintaining the viability of the virus over time is achieved.

Reference

1. Petri et al. Surg Clin North Am 2003; 83:597–616.

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(4) A Model for Studying the Influence of Mechanical Loading on Human Periosteal Stem Cells

Bonzani I.C.¹, Knight M.M.², Lee D.A.², Bader D.L.², Williams A.³, Stevens M.M.¹

¹Department of Materials, Imperial College London, London SW7 2AZ, United Kingdom

²IRC in Biomedical Materials, Queen Mary University London, London E1 4NS, United Kingdom

³Chelsea and Westminster Hospital NHS Trust, London SW10, United Kingdom

Due to their inherent regenerative potential, much research is now focused on using adult stem cells in numerous tissue engineering applications. The future success of such therapies relies on the ability of scientists to identify stem cell sources and subsequently isolate and control the differentiation of adult stem cells *in vitro*. Recently, the periosteum has been identified as a stem cell source in various animal models, with the ability to form osteogenic and chondrogenic tissue types. To date, however, the presence and features of this stem cell population within human periosteum have not been fully characterised. We have successfully isolated and expanded a population of stem cells within human periosteal explants and characterised their growth potential, lineage specificity, and gene expression. We have also investigated the use of external mechanical stimulation as a means to induce proliferation and differentiation pathways within the periosteal explants. Utilising a well-characterised mechanical loading system (1) and established image-analysis techniques (2) we subjected periosteal explant-agarose gels to a range of loading regimes for periods of up to 4 days. Changes in cell proliferation and differentiation were determined by analysing metabolic activity and gene expression [real time RT-PCR]. This model system describes, for the first time, the influence of mechanical stimulation on stem cells within human periosteal explants.

References

1. Knight MM, Ghorri SA, Lee DA et al. (1998). Measurement of the deformation of isolated chondrocytes in agarose subjected to cyclic compression. Med Eng Phys 20(9): 684–688.
2. Lee DA and Knight MM. (2004). Mechanical loading of chondrocytes embedded in 3D constructs: *in vitro* methods for

assessment of morphological and metabolic response to compressive strain. Methods Mol Med 100: 307–324.

(5) A New Generation of (Bio)capsules: Lyophilisomes

†Geutjes P., †Daamen W., van Moerkerk H., Nillesen S., Wismans R., Hafmans T., van den Heuvel P., Pistorius A., Veerkamp J., van Hest J., van Kuppevelt T.

Department of Biochemistry 280, NCMLS, Radboud University Nijmegen Medical Centre, P.O. Box 9101, Nijmegen 6500 HB, The Netherlands

†These authors contributed equally to this work.

Background: The preparation of capsules from biological macromolecules offers a major challenge in biomaterials science. With this simple and new technique, capsules can be prepared with molecules of interest in the capsule wall and/or lumen. This new generation of capsules have high potency in the field of drug delivery and vaccination.

Methods: Capsules were obtained by quench freezing an elastin solution followed by lyophilisation according to a standardised procedure. The morphology was analysed by scanning and transmission electron microscopy. Fluorescent probes were used to evaluate the incorporation in the capsule wall and lumen and its release.

Results: Capsules ranged from 200 nm–10 µm and fluorescent-labeled (macro)molecules could be differentially incorporated in the capsule wall and lumen and were released by elastase digestion. Capsules could also be prepared from other proteins (serum albumin and atelocollagen) and from a sugar (heparin).

Discussion: We developed a new type of capsules which we call ‘lyophilisomes’. The methodology comprises three phases: micro-phase separation by fast freezing, structural rearrangement by annealing and the creation of a lumen by lyophilisation, most likely according to a 3D analogy of the coffee stain principle. Capsules could be prepared from various macromolecules, and capsule formation does not rely on amphiphilicity. Since this is a mild technique, molecules of interest e.g. antibodies and enzymes may be incorporated in and subsequently released from the lumen and/or wall of the capsules. Lyophilisomes thus have potential for targeted drug delivery.

(6) A Novel Bioreactor for Three-Dimensional Culture of Engineered Cardiac Tissue

Wang J., Liu T., Song K., Sun X.

Dalian Research and Development center for Stem Cell and Tissue Engineering, State Key Laboratory of Fine Chemicals, Dalian University of Technology, Liaoning, Dalian, 116024, China

Mechanical strain and electrical stimulation play important roles in fabricating engineered heart tissue *in vitro*. By properly controlling the parameter of stimulation, the cellular viability and metabolism could be remarkably promoted and the engineering heart tissue

could spontaneously pulsate, which can meet the increasing demand of engineered cardiac tissue used in clinical transplantation.

We have developed a novel bioreactor to investigate the effect of mechanical strain and electrical stimulation on fabrication of engineered heart tissues *in vitro*. Cardiac myocytes freshly isolated from neonatal SD rats were mixed with neutralized collagen sponge and inoculated into the bioreactor. Frequency pressure was given to the resilient chamber walls to simulate the kinestate of ventricular wall. Electrical stimulation was exerted to the engineered heart tissue by adding voltage with certain frequency to the rheophore. Thus the microenvironment *in vivo* was simulated to enhance the viability of cardiac myocytes and cause the contraction synchronously.

The results showed that the cardiac myocytes adhered to the collagen sponge presented good phenotype. Under the stimulation of mechanical strain, the cardiac myocytes were well-distributed throughout the constructs. With the stimulation of electrical signal, cardiac myocytes got synchronous contraction, cellular metabolism prospered and the viability was high.

It is concluded that the engineered myocardial tissue fabricated in our bioreactor could maintain high biologic activity. The direction of cellular spread is homogeneous and the cardiac myocytes are well-distributed. The novel bioreactor contains pulsatile perfusion, mechanical strain and electrical stimulation, with which a mimic environment can be provided to engineer heart tissues *in vitro*.

Key words: Cardiac tissue engineering; Mechanical strain; Electrical stimulate; Bioreactor

(7) A Novel Model for the Investigation of the Fate of Tendon Grafts

Alam N.H., Wong J.K., McGrouther D.A.

University of Manchester, Department of Plastic and Reconstructive Surgery Research, Stopford Building, Oxford Road, Manchester, M13 9PT, United Kingdom

Research is underway by a number of tissue engineering groups to develop a tissue-engineered tendon substitute that may have better and quicker healing properties. How a tendon graft heals will provide much needed guidance in developing strategies for tissue-engineered tendon. We have devised a novel tendon graft model in a mouse, which may be useful to study the integrative properties of a tendon construct.

Using techniques originally used by Wong *et al.* (1) in mice flexor tendons we have characterised and developed a mouse Achilles tendon model to study the fate of tendon engraftment. The cellular population and the extracellular matrix of Achilles tendon were studied using H&E, Masson's Trichrome (collagen), Alcian blue (fibrocartilage), Miller's stain (elastin) and TRITC-phalloidin (cellular cytoskeleton). Vascularisation of the tendon was investigated using FITC-labelled dextran perfusion studies and antibodies to CD 31 (2).

We have established that the mouse Achilles tendon provides a suitable site to investigate the biology of tendon engraftment. The architecture, vascularity and elastin content indicate that the tendon is extrasynovial in nature. It is feasible through microsurgical technique to perform reliable tendon grafts in this region; furthermore it has the versatility to investigate tissue engineered construct viability.

References

1. Wong J., Bennett W., Ferguson M.W.J., McGrouther D.A. (2006) Microscopic and histological examination of the mouse hindpaw digit and flexor tendon arrangement with 3D reconstruction. *J Ant.* 209(4): 533–545.
2. O'Ceallaigh S., Herrick S.E., Bluff J.E., McGrouther D.A., Ferguson M.W. (2006). Quantification of total and perfused blood vessels in murine skin autografts using fluorescent double-labelling technique. *Plast Reconstr Surg.* 117(1): 140–151.

(8) A Novel Poly(L-Lactide-co-ε-Caprolactone)-Collagen Hybrid Construct for Application in Tissue Engineering

Ananta M.¹, Hilborn J.², Aibibu D.³, Brown R.A.¹, Mudera V.¹

¹UCL Tissue Repair & Engineering Centre, Institute of Orthopaedics, Stanmore Campus, HA7 4LP, United Kingdom

²The Ångström Laboratory, Department of Materials Science, Uppsala University, P.O. Box 534, 751 21 Uppsala, Sweden

³Institut für Textiltechnik, University of Technology Aachen, Eilfschornsteinstraße 18, 52062 Aachen, Germany

A biodegradable hybrid construct consisting of a slow degrading poly(L-lactide-co-ε-caprolactone) (PLA-ε-CL) knitted mesh, plastically compressed (1) between two collagen gels was fabricated and tested *in vitro* for tissue engineering applications. The polymer mesh was incorporated to give greater mechanical stability to the compressed collagen scaffolds.

The hybrid construct was characterized for fluid (weight) loss and cell viability during compression and mechanical properties. Hybrid constructs embedded and surface layered with human dermal fibroblasts (2, E + 5 per 5 ml) were cultured for up to one week in static culture. Quantitative and qualitative data on cell viability and proliferation were obtained.

It was found that the fluid (weight) loss in plastic compression of the hybrid construct was time dependent and not weight dependent at an applied load of 240 grams. No significant cell death was observed during the plastic compression process and a homogenous cell distribution was achieved. One week of static culture showed that the cultivated hybrid construct retained its mechanical properties with no evidence of degradation, and cells inside the constructs as well as layered on top of the constructs proliferated.

We found the PLA-ε-CL-Collagen hybrid construct a useful three-dimensional scaffold for tissue engineering of stratified tissues and potential applications in bladder wall, blood vessels and skin are currently being explored.

Reference

1. R. A. Brown, M. Wiseman, C-B. Chuo, U. Cheema and S. N. Nazhat. Ultrarapid engineering of biomimetic materials and tissues. *Adv. Funct. Mater.*, 2005, 15, 1762.

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(9) A Study on Biopolymers for Tissue Engineering Applications with Regard to Nerve Regeneration

Stark Y., Bruns S., Kasper C., Stahl F., Scheper T.

Institute for Technical Chemistry, University of Hannover, Calinstr.3, 30167 Hannover, Germany

The number of recently developed functional scaffolds for tissue engineering and likewise peripheral nerve regeneration is large. Until now the ideal physico-chemical compositions of such materials and the ideal surface structure and functionalisation have not yet been found. Although peripheral nerves show capacity for regeneration after injury, the regeneration depends on the type of the gap and the length of the gap. Nerve regeneration competes with formation of scar tissue, without appropriate interventions, and results in a permanent loss of peripheral nerve regeneration. The successful repair and regeneration of tissue by tissue engineering depend on the biocompatibility and biodegradability of the used biopolymers. Furthermore, scaffold material has to fulfil different requirements, and should support cell attachment, proliferation and differentiation to the desired phenotype.

The aim of the study was to evaluate and characterise different biopolymers like collagen I and the polysaccharide polysialic acid for tissue engineering applications. The biopolymers were modified to investigate structured surfaces which could enhance cell growth. The scaffold-cell interactions were studied using model cell lines. Moreover, different cell markers were tested by RT-PCR studies.

Biocompatibility studies with Hep-G2 cells revealed no significant cytotoxicity of the polysialic acid and its modifications. The neurological cell line PC-12 and the immortalised Schwann cells had a high viability and proliferation rate. The RT-PCR results showed the expression of the cell marker tyrosine hydroxylase and the house keeping gene GAPDH. PC-12 cells could be differentiated neurologically on the tested materials.

(10) A Tissue Engineering System for *In Vitro* Mechano-Stimulation and Material Evaluation

Kessler O., Sommers M., Bottlang M.

Stryker Orthobiologics, Florastr. 13, CH 8800 Thalwil, Switzerland
Legacy Clinical Research & Technology Center 1225 NE 2nd Avenue Portland, OR 97232, United States of America

Introduction: We developed a mechano-active tissue engineering (MATE) system which combines the capabilities of delivering controlled mechano-stimulation and assessing the development of material properties *in vitro* like articular cartilage specimens and hydrogel scaffolds.

Methods: The MATE system subjects scaffolds to dynamic unconfined compression under load control. Scaffolds are maintained in six individual culture dishes on the actuator box. Six actuators generate an upward-directed uniaxial force that compresses each scaffold against flat posts contained in the culture lid. For material property characterization, the amount of compression is assessed individually for each culture by six linear encoders with 1 μ m resolution. The MATE system was employed to measure the equilibrium modulus ME and the dynamic modulus MD of hydrogel specimens (6 mm, $n = 3$) and bovine articular cartilage explants (4.5 mm, $n = 3$). ME was assessed in terms of the accumulative strain in response to a static compressive load of 1 N (0.5 N preload) over 5 minute duration. MD was assessed in terms of the strain

amplitude in response to a dynamic load amplitude of 1 N (0.5 N preload) applied at 10 Hz.

Results: Articular cartilage specimens yielded pronounced viscoelastic behavior, with a more than 20-fold difference between ME = 0.65 ± 0.11 MPa and MD = 15.7 ± 0.9 MPa. Hydrogel specimens yielded significantly lower moduli (ME = 0.15 ± 0.01 MPa) and MD = 0.26 ± 0.01 MPa. These results demonstrate that the MATE system is capable of both mechano-stimulation by dynamic compression, and material property evaluation *in vitro*.

Conclusion: The MATE system provides for controlled application of mechano-stimulation and sensitive assessment of dynamic material properties crucial for cartilage function.

(11) Adipocyte Differentiation Induced Using Non-specific siRNA Controls in Cultured Human Mesenchymal Stem Cells

Xu Y., Mirmalek-Sani S.H., Lin F., Zhang J., Oreffo R.O.C.

University of Southampton, Bone and Joint Research Group, School of Medicine, Southampton SO16 6YD, United Kingdom

RNA interference (RNAi), mediated by short interfering RNA (siRNA), has emerged as a powerful tool to suppress gene expression in mammalian cells, with potential for developing novel treatments. However, recently, the specificity of RNAi has been questioned with emergent evidence of off-target activity in mammalian cells. We have examined the RNAi off-target activity of a commercial negative siRNA resulting in the induction of adipogenesis in human fetal femur-derived mesenchymal stem cells (fetal MSCs) and in human pre-adipocyte cells. We examined a non-specific siRNA control on fetal MSCs using morphology and histochemistry and quantitative real-time PCR (qRT-PCR) to determine the effects on adipogenesis. Application of a non-specific control siRNA (0 to 80 nM) resulted in a dose-dependant enhancement of adipogenesis in fetal MSCs. This was confirmed by quantitation of adipocyte number between non-specific siRNA group and mock transfection group (siRNA used at 10 nM, $p < 0.05$; at 80 nM, $p < 0.01$). In addition, expression of PPAR γ -2, a marker of adipogenesis, was observed in cells transfected with the non-specific siRNA at the day 7 of adipogenic induction by qRT-PCR, when no induction was observed in cells subjected to mock transfection. Examination of the osteogenic gene, Runx2, using qRT-PCR in fetal MSCs transfected with the non-specific siRNA showed no effect and absence of sensitivity of the MSCs to siRNA/lipid mixture. Our results raise concerns as to the specificity of RNAi in mesenchymal stem cells, indicating a need for caution and a critical need to understand the rules governing specificity of siRNA, particularly where therapeutic regimes are advocated and permanent RNAi is to be established.

(12) Adipogenic Differentiation Increases the Angiogenic Capacity of Human Adipose-Derived Stromal Cells

Verseijden F., Jahr H., Posthumus-Van Sluijs S.J., Van Neck J., Van Osch G., Hofer S.O.P., Erasmus M.C.

University Medical Center, Department Plastic & Reconstructive Surgery, Rotterdam, P.O. Box 2040, 3000 CA, The Netherlands

Improving vascularization of engineered adipose tissue constructs is a major challenge in the field of plastic surgery. Although both differentiated adipocytes and undifferentiated adipose-derived stromal cells (ADSC) are known to release angiogenic factors, the optimal stage of differentiation of ADSC for *in vivo* implantation is not known. To estimate this stage, we studied the expression and secretion of a large panel of angiogenic factors during ADSC differentiation. Furthermore, we evaluated the ability of the conditioned medium to stimulate endothelial cell (EC) growth.

Passage 1 or 2 human ADSC were plated at a density of 0.2×10^5 cells/well in 6-well plates and cultured on adipogenic medium or control medium. Cell and medium samples were taken at day 0, 3, 7, 14 and 22. The stage of adipogenic differentiation of the hADSC was assessed using Oil Red O staining, gene expression of FABP4 and GPDH activity.

The secretion of VEGFA, PLGF, ANG-1 and -2 significantly increased during adipogenic differentiation of the ADSC until day 14. Moreover, ECs cultured in conditioned media of human ADSC differentiated for 3, 7 and 14 days had significantly higher growth rates ($122\% \pm 8.5\%$) than ECs cultured in conditioned medium of undifferentiated human ADSC ($92\% \pm 5\%$).

We conclude that adipogenic differentiation of human ADSC *in vitro* improves their angiogenic capacity. Our results suggest that human ADSC differentiated for a period of 1 to 2 weeks will give optimal angiogenic responses for *in vivo* implantation.

(13) Aligned Nanofibrous Scaffold Dictates Directional Cellular Growth

Dunning M.¹, Henry J.A.^{1,2}, Casey R.G.³, Pandit A.^{1,2}

¹Department of Mechanical and Biomedical Engineering,

²National Centre for Biomedical Engineering Science, ³Department of Urology, University College Hospital Galway, National University of Ireland, Galway, Ireland

The optimal tissue engineering scaffold is one which replicates the native tissue in terms of architecture and mechanical properties. Functional scaffolds that mimic properties of native tissues may be designed by structurally aligning fibre networks during fabrication. Ideally, porous networks should incorporate structural cues to transmit guidance cues throughout tissue development. Electrospinning was used to fabricate nanofibrous poly(ϵ -caprolactone) scaffolds of varying architecture by modifying the rotating target speed and polymer concentration. The target speed varied between 400 rpm–1000 rpm, with the polymer concentrations between 8–12%. The graft prototype architecture was characterised using images from scanning electron microscopy (SEM). Custom designed software was used to quantify fibre alignment. To study cellular alignment, fibroblasts cells were seeded at a density of 10×10^4 cells onto each scaffold. Cell alignment was examined at 1d, 3d and 7d using a fluorescent phalloidin stain and SEM images. Mechanical properties including ultimate tensile strength, Young's modulus and strain to break were determined with uniaxial tensile testing. The longitudinal and transverse directions were tested due to their anisotropic nature. Results indicate that target rotation speed influenced fibre alignment, with 400 rpm inducing a random fibre orientation, and greater than 750 rpm producing parallel aligned fibres. Aligned fibres imparted orthotropic properties to the networks. Fibre diameter increased with polymer concentration.

Florescent microscopy and SEM images revealed parallel fibroblast cell growth to the aligned fibres. In conclusion, aligned scaffold fibres dictated directional cellular growth.

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(14) An Efficient Animal Model for Studying Angiogenesis of Engineered Constructs: Octet Truncal Pedicles of Rabbit

Demirtas Y., Engin M.S., Cifci M., Karacalar A.

Ondokuz Mayıs University Medical School, Department of Plastic, Reconstructive and Aesthetic Surgery, 55139 Samsun, Turkey

Introduction: Small animal models used for assessment of vascular pedicle derived *in vivo* vascularization of the engineered constructs are scarce and allow at most two experiment sites in a single animal. The purpose of this study is to develop a more feasible small animal model.

Materials and Methods: Ten rabbits were used for the study. Anatomic dissections were done in five and angiographic studies were performed in the others. Ascertained pedicles were evaluated for pedicle length, ease of dissection and potential area of supply for implantation of 3D constructs.

Results: The trunk of the rabbit is supplied by 8 vascular pedicles on either side. These are directly visible, easily accessible, and similar in size. Also, they comprise adjacent angiosomes, providing a suitable setting for researching the interplay between vascular structures and response to premeditated conditions. Each measure averagely 8 centimeters, and emerge as a large vessel and taper off, providing a pedicle of versatile caliber.

Discussion: Anatomical consistency of these vessels was demonstrated by the senior author previously, and as confirmed by our findings, it is possible to make 8 experiments on single animal with this model. This is cost effective, and amenable to conduct multiple experiments on same subject, increasing the dependability of the findings. Due to accessibility and vital insignificance of these structures, specimens can be harvested without killing the subjects, making them available for further use. Thus, this model boasts advantages over other models regarding cost and technical challenge. This model has been, and is still being used in a number of studies, yielding satisfactory results.

Conclusion: We believe that octet truncal pedicle model is an optimal model for tissue engineering studies which dwell into angiogenesis.

(15) An *In Vitro* Endochondral Ossification Model Using Human MSCs

Van der Jagt O.P., Koevoet J.L.M., Kops N., Hellingman C.A., Jahr H., Weinans H., Van Osch G.J.

Orthopaedic Research Laboratory, Erasmus MC, Room EE-1614 P.O. Box 2040, 3000 CA Rotterdam, The Netherlands

Objective: In order to examine endochondral ossification *in vitro* in human mesenchymal stem cells (hMSCs), we examined the influence of different combinations of TGF-beta, beta-glycerol-phosphate and dexamethasone on chondrogenic differentiated MSCs. We were interested what condition resembled the temporal pattern of endochondral related proteins, mineralisation and the expression of chondrogenic and osteogenic genes.

Methods: After 3 or 5 weeks of chondrogenic differentiation of human MSCs, pellet cultures were cultured for two more weeks on different media containing different combinations of TGF-beta, beta-glycerol-phosphate and/or dexamethasone. After 2, 3, 5 and 7 weeks pellets were stained with monoclonal antibodies against collagen type 2 (Col-2), collagen type 10 (Col-10) and osteocalcin and were stained for mineralisation. Gene-expression was analysed using RT-PCR for Sox-9, Col-2, Col-10, alkaline phosphatase, VEGF and HIF-1a.

Results: Marked collagen type 2 staining was seen at 3 weeks and this increased in time. Collagen 10 staining was seen from 5 weeks and was more present when the medium was switched. Calcium phosphate depositions were only in beta-glycerol-phosphate containing medium and more pronounced when the medium did not contain TGF-beta. A switch to osteogenic medium, containing beta-glycerol-phosphate and dexamethasone, downregulated Sox-9 and Col-2 gene expression whereas Col-10 and Alp were upregulated.

Conclusions: Micromass cultures of hMSCs can serve as an *in vitro* model to examine endochondral ossification as they show the same temporal pattern as in the growth-plate (chondrogenic differentiation-hypertrophy-mineralization). Our data suggests that five weeks of culture in chondrogenic medium and then switch cultures to osteogenic medium gives the highest amounts of matrix proteins and mineralisation.

(16) Analysis and Control of Process Conditions for the Fabrication of Supercritical Fluid Foamed Poly(D,L-Lactic Acid) Scaffolds

White L.J.¹, Howdle S.M.², Shakesheff K.M.¹

¹School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, United Kingdom

²School of Chemistry, University of Nottingham, Nottingham, NG7 2RD, United Kingdom

Morphology of tissue engineering scaffolds is integral to the formation of tissue and controlled delivery of incorporated growth factors. Clinical applications require reproducible pore size and structure of scaffolds. The production of scaffolds using supercritical carbon dioxide (scCO₂) involves three stages: (1) the granular polymer is plasticized in the presence of scCO₂, (2) scCO₂ molecules diffuse into the plasticized melt and (3) upon depressurization, gas bubbles nucleate and grow which form the resultant pore structure. This complex process can produce scaffolds of divergent pore size and structure. This study sought to elucidate the effect of processing conditions and molecular weight on scaffold morphology.

Three molecular weights of poly(D,L-lactic acid) (PDLLA) (18KD, 28KD and 52KD) were used to form scaffolds under dif-

ferent processing conditions. The parameters investigated included temperature (from 25°C to 45°C) and depressurization rate. The morphology of the resultant scaffolds was characterized by micro X-ray computed tomography (microCT) and scanning electron microscopy (SEM) respectively.

The porosity of the scaffolds significantly increased with increased venting times (decreased depressurization rates). This was most clearly observed with the low (18KD) and high (52KD) molecular weights of PDLLA. The porosity increased from 78% for the 18KD PDLLA (64% for the 52KD PDLLA) at a vent time of 5 minutes, to 90% (77% for the 52KD PDLLA) at a vent time of 120 minutes. At each vent time, depressurization from 230 bar occurred at a constant rate. In conclusion, the morphology of PDLLA scaffolds can be tailored by adjusting the processing conditions and the molecular weight of the polymer.

(17) Anatomical Study for Small Animal Cartilage Defect Model

Saha S.¹, Kirkham J.¹, Wood D.¹, Curran S.² and Yang X.B.¹

¹Department of Oral Biology, University of Leeds, Leeds LS2 9LU, United Kingdom

²Smith & Nephew Research Centre, York Science Park, Heslington, York YO10 5DF, United Kingdom

The gold standard for validating novel treatment strategies in tissue engineering is via *in vivo* models. This allows tissue-specific maturation and integration to permit potential achievement of appropriate tissue architecture and function. A number of cartilage repair models have been developed in larger animals. However, they are not suitable for testing treatments involving the use of human cells due to problems of immune rejection. The use of smaller (immunodeficient) animals would overcome this limitation. Aims: site specific anatomical dissection of rodents (mouse & rat) and rabbits (control) to identify cartilaginous areas to inform the choice of defect sites. Sites were prepared under a dissecting microscope. Rib, xiphoid process, femoral condyle and patellar groove cartilage surfaces were measured with a digital dial caliper. Results: the lengths and widths were: 1) medial condyle: mice (1.53 mm and 0.995 mm); rats (4.38 mm and 2.58 mm); rabbit (11.8 mm and 6.26 mm); 2) lateral condyle: mice (1.61 mm and 0.93 mm); rats (4.35 mm and 2.57 mm); rabbit (11.98 mm and 6.25 mm); 3) patellar groove cartilage surfaces: mice (2.37 mm and 1.21 mm); rats (6.18 mm and 2.88 mm); rabbit (17.18 mm and 7.14 mm); 4) xiphoid process: mice (3.26 mm and 0.58 mm), rats (5.69 mm and 1.29 mm); rabbit (14.73 mm and 2.82 mm); 5) rib: mice (4.27 mm and 0.38 mm), rats (9.93 mm and 1.06 mm); rabbit (23.23 mm and 1.92 mm). Conclusion: This study provides anatomical information for creating a suitable *in vivo* model in rodents to accommodate differences in the type of cartilage to be regenerated and variations in scaffold type.

(18) Angiogenic Stimulation by Fibrin-E: The Basis of a Smart Matrix Dermal Scaffold

Dye J.F., Baldwin C.J., Potter M., Patel N., Almqvist S., Edwards F.C., Di Y., Rolfe, K.J., Clayton L., Irvine L.M., Linge C.

RAFT Institute, The Royal Free Hospital NHS Trust, Mount Vernon Hospital, Northwood, Middlesex, HA6 2RN, United Kingdom

Fibrin and its plasminolytic degradation products have been recognised to stimulate angiogenesis. The harnessing of this property in a tissue engineered dermal scaffold by using crosslinking chemistry is under development in our laboratory. The potency of such scaffolds, in contrast to current clinical materials, is shown by rapid ingress of endothelial cells *in vitro*. However the mechanisms involved in pro-angiogenic effects of fibrin are unclear.

Recent data demonstrate that human microvascular endothelial cells (HPMECs) migrate more through mixed fibrin collagen gels than gels of collagen alone. The soluble fibrin-E fragment potentially recapitulates the effect of whole fibrin admixed with collagen. HPMEC adhesion to fibrin and fibrin-E is blocked by GRGDS pentapeptide and substantially mediated by α -v integrins, but not by α -5 β 1 or α -2b β 3. The conserved A-chain 95 RGD in the rod region falls outside the disulphide knot domain and is not only downstream of a potential plasmin cleavage site but is a possible cleavage site itself. Therefore, whether fibrin-E carries an RGD motif is unresolved. HPMEC binding to gelatin is entirely RGD and α -v-dependent. Fibrin-stimulated migration is ablated by VEGF-blocking antibody and a specific VEGF-r2 inhibitor. Exposure of HPMECs to fibrin or fibrin-E shows an increase in intracellular VEGF mRNA and protein at 24 hr.

Fibrin-E, a stable disulphide knot domain found to resist proteolysis, released by degradation of fibrin-based matrices could exert a local pro-angiogenic stimulation to reinforce endothelial cell interactions with the scaffold material itself. These data suggest a novel smart matrix mechanism.

(19) Assessment of Scaffold Guided Tissue Regeneration in Full-Thickness Wounds in Rabbits

Eweida A., Marei M., Gabr E., Rafik M.

Alexandria University, Egypt

We compared the conventional Wolfe's skin graft with skin regeneration using porcine UBM scaffolds either alone or with cultured keratinocytes. Full thickness skin wounds were created on the back of NewZealand rabbits and classified into three groups, the first treated with autogenous full thickness skin graft (Wolfe's), the second treated with UBM, the third treated with UBM with cultured keratinocytes. The wounds were examined for graft take, wound contracture, pigmentation, and histology, at first, second and third weeks of implantation. In our pilot study we found that the results of scaffold guided tissue regeneration using UBM scaffolds were very close to the conventional Wolfe's graft. Histological assessment revealed regeneration of nearly all skin layers by three weeks with regeneration of skin appendages.

(20) Attachment and Function of Corneal Endothelial Cells after Transplantation

Bednarz J.¹, Aintablian A.¹, Rousselle P.²

¹University Medical Center Hamburg-Eppendorf, Department of Ophthalmology, Hamburg, Germany

²Institut de Biologie et Chimie des Protéines, CNRS/Université Claude Bernard Lyon 1 UMR5086, IFR 128 Biosciences Lyon-Gerland, Lyon, France

Most tissues are covered by a tight cellular layer. This cell layer serves as a barrier but can also have physiological functions. In the

case of the corneal endothelium, the cells of this monolayer regulate hydration and thereby transparency of the corneal stroma. The aim of our study was to investigate on the one hand the attachment of corneal endothelial cells to the underlying matrix and on the other hand the formation of intercellular connections. For the first time we demonstrated the presence of laminin-5 in the basement membrane of human corneal endothelial cells (Descemet's membrane). Cell attachment analysis showed that laminin-5 coating of culture dishes facilitates attachment of human corneal endothelial cells and that this effect can be neutralized by a monoclonal antibody against laminin-5. Electron microscopic analysis of cultured human corneal endothelial cells revealed cell polarity and intercellular junctions comparable to those found in the endothelial cell layer of human donor corneas. Immunohistochemical staining showed the presence of ZO-1, a tight junction associated protein, between the human corneal endothelial cells. In summary our results showed that attachment of human corneal endothelial cells can be improved by coating the surface with laminin-5. Furthermore, the self organisation of the cells leads to a tight endothelial cell layer providing the basis for a functional human corneal endothelium.

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(21) Automated Rapid Tissue Fabrication Device: Prototype and Proof of Principle

Alp B.¹, Blunn G.W.², Hanson B.³, Brown R.A.¹

¹University College London, Tissue Repair and Engineering Centre, ²Centre for Bio-Medical Engineering, Institute of Orthopaedics, Stanmore Campus, London, HA7 4LP, United Kingdom, ³University College London, Mechanical Engineering, WC1E 7JE, United Kingdom

Tissue engineering for some time has aimed to fabricate useful 3D connective tissue substitutes through culturing cells in bioreactors. However, even the best examples of extracellular matrix synthesis onto scaffolds have proved to be very slow (costly) and poorly biomimetic. We recently developed a rapid microfabrication technique in which native, tissue dense collagen-cell constructs can be prepared in minutes with highly controllable, complex microstructure. This relies on expulsion of fluid from collagen-cell hydrogels by compression. The present study has produced a semi-automated tissue fabrication device capable of continuous production of tissue-density collagen sheets suitable (for example) as dermal substitutes.

Collagen gels are cast on a moving belt in a controlled environment chamber prior to compression by a powered plunger, expelling fluid into porous layer. At the end of the first casting belt, gels transfer to a porous surface belt for compression. Compressed collagen sheets (~50 μ m thick) are collected on top of each other as a multilayer constructs. All aspects of the process, the composition/density/cell type in each layer are controllable through manipulation of the computer regulated rates, pumping stages and compression times. This device represents proof-of-principle of a fully controllable automated rapid tissue fabricator, suitable for production of implants at the bedside.

References

1. Cheema et al. (2007) *Biotechnology and Bioprocessing Engineering* 12:9–14.
2. Brown et al., (2005) *Advanced Functional Materials* 15:1762–1770.

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(22) Bacterial Cellulose: A New Biomaterial for Mucosal Regeneration of Trachea

Suehiro A., Kanemaru S., Umeda H., Amano Y., Omori K., Ito J.

Department of Otolaryngology, Head & Neck Surgery, Graduate School of Medicine, Kyoto University 54, Kawaharacho, Shogoin, Sakyo-ku, Kyoto, 606-8507, Japan

Our group have tried to regenerate upper respiratory tracts with tissue engineering technique and succeeded it by using polypropylene mesh covered with fascia. Though, collecting fascia needs another surgical treatment, new biomaterial alternate for fascia has been desired. Bacterial cellulose (BC) is applied in many fields today, but not yet in tissue engineering field. Helenius G, *et al.* demonstrated the good biocompatibility of BC *in vivo*. So, we evaluated the potential of BC as an alternate material for fascia for mucosal regeneration of the trachea in canine experiments.

Experiment 1: A round defect (1.5 cm in diameter) was created on the cervical trachea. A non-treated BC membrane was sutured to the defect. The implanted membrane dropped off because of local infection about 15 days after implantation.

Experiment 2: Fibroblasts harvested from subcutaneous tissue were cultured on polarized BC and non-treated BC *in vitro*. More remarkable proliferation was observed on polarized BC than non-treated BC.

Experiment 3: The fibroblasts-attached BC made in Experiment 2, was used in this experiment. This “cell sheet BC” was implanted 20 days after cell seeding with the same procedure as Experiment 1. The surface of the sheet was covered with extended mucosa without local infection.

This study demonstrated that “cell sheet BC” was a useful biomaterial for regeneration of the tracheal mucosa. And this cell sheet like method was very convenient because it didn't have the necessity of cell detachment from culture membrane. This tissue engineering technique was a possible treatment for regeneration of tracheal defect.

(23) Balanced Co-expression of VEGF164 and PDGF-BB Prevents Aberrant Angiogenesis and Improves Hind-Limb Ischemia

Banfi A.^{1,2}, Von Degenfeld G.¹, Merchant M.J.¹, McDonald D.M.³, Blau H.M.¹

¹Stanford University, Baxter Laboratory in Genetic Pharmacology, Stanford, United States of America

²Basel University Hospital, Departments of Surgery and of Research, Basel, Switzerland

³UCSF, Department of Anatomy, San Francisco, United States of America

VEGF regulates normal vessel growth in development and physiological angiogenesis. However, VEGF gene delivery can have serious dose-dependent side-effects, such as the appearance of aberrant vascular structures similar to cavernous angiomas. We have recently found that the switch between induction of stable capillaries and progressive angioma growth occurs at a threshold level in microenvironmental VEGF concentration and correlates with aberrant pericyte recruitment. Since pericytes are responsible for the maturation of nascent vessels and are recruited by PDGF-BB, we investigated the role of PDGF-BB signaling in modulating VEGF-induced angiogenesis.

Primary mouse myoblasts were retrovirally transduced to deliver either murine VEGF164 or human PDGFb to skeletal muscle. Although PDGF-BB did not induce any angiogenesis, its co-expression significantly increased VEGF-induced vascular density and prevented aberrant vessels, yielding a network of homogeneous, mature capillaries. However, both factors required precise co-localization in the microenvironment at fixed relative levels, through co-expression from a single bicistronic retrovirus. In a mouse model of hindlimb ischemia, balanced co-expression greatly improved blood flow, stimulated collateral arteriogenesis and reduced muscle damage compared to VEGF or PDGF-BB alone. PDGF-BB did not influence the initial response to VEGF164, but rather modulated subsequent remodeling, preventing circumferential enlargement and leading to capillary sprouting. Mechanistically, we found that the VEGF level, at which the threshold between normal and aberrant angiogenesis occurs, was not fixed, but was determined by the balance between VEGF and PDGF-BB signaling.

Our results suggest PDGF-BB-mediated vascular maturation can be a complementary target to both normalize and potentiate VEGF-induced angiogenesis.

(24) Behavior of Implanted Bone Marrow Derived Stromal Cells

Kanemaru S.¹, Yamashita M.¹, Umeda H.¹, Ohono T.¹, Suehiro A.¹, Hirano S.¹, Tamura Y.¹, Omori K.², Nakamura T.³, Ito J.¹

¹Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University

²Department of Otolaryngology, Fukushima Medical University School of Medicine

³Department of Bioartificial Organs, Institute for Frontier Medical Sciences, Kyoto University

Introduction: Mesenchymal stem cells (MSCs) are pluripotent cells that have the potential to differentiate into cells of different lineages. We previously reported successful regeneration of an injured vocal fold in a canine model by implantation of BSCs containing MSCs. However, the fate and the behavior of the implanted BSCs were not identified. It is an important issue to trace implanted BSCs to distinguish what types of tissues they become at the injected side of the vocal fold. The aim of this study is to investigate the destiny and the behavior of implanted autologous BSCs.

Materials and Methods: After harvesting bone marrow from the femora of adult beagle dogs, adherent cells were cultured and selectively amplified. These BSCs were infected by GFP (green fluorescent protein) transferred Lentivirus and they were injected into an injured vocal fold. Immunohistologic examination of the resected vocal folds was performed 12 weeks after the treatment. BSCs also marked by BrdU (Bromodeoxyuridine) were injected into an injured vocal fold and were examined how long were they able to detect *in vivo*.

Results: BrdU-labeled BSCs were able to detect over 2 weeks after implantation. Implanted cells were alive in the host tissues and a part of them showed positive expression for keratin and desmin, which are markers for epithelial tissue and muscle, respectively.

Conclusion: Implanted BSCs have the possibilities not to proliferate but to differentiate into more than two tissue types *in vivo*. They may contribute to tissue regenerations by paracrine and/or autocrine.

(25) Benzylaminated Dextran Modified Hydrogels: A TGF- β 1 Carrier for Tissue Engineering?

Degat M.C., Dahri-Correia L., Lavigne F., Meunier A., Petite H., Sedel L., Logeart-Avramoglou D.

Laboratoire de Recherches Orthopédiques, CNRS UMR 7052, Faculté de médecine Lariboisière Saint-Louis, Paris, 75010, France

To elicit long term retention of TGF- β 1 *in situ*, dextran-based hydrogels (in which a benzylaminated dextran (DMCB) exhibiting high affinity for TGF- β 1 was immobilized) were developed.

A range of hydrogels were prepared by co-crosslinking native dextran (500 or 2,000 KDa) and various amounts of DMCB (ranging from 0 to 16.9% w/w) with sodium trimetaphosphate. *In vitro* TGF- β 1 release from hydrogels was monitored over 18 hours and 3 weeks. Retained TGF- β 1 bioactivity after 3 weeks released kinetics was determined using a cells assay.

While unmodified hydrogels rapidly desorbed 80–90% of the preloaded TGF- β 1, DMCB-hydrogels desorbed only 40–60% of TGF- β 1 over 21 days in PBS. Increasing the DMCB ratio from 6.6 to 16.9% did not affect the TGF- β 1 retention. Release experiments performed in high ionic strength solutions (from 0.15 M to 1.5 M NaCl) indicated that formation of the complex between TGF- β 1 and functionalized hydrogels was governed by different interactions depending on the degree of conjugation with DMCB (specifically, ionic for weakly conjugated matrices while non-ionic for highly conjugated matrices). The bioactivity of sequestered TGF- β 1 was confirmed using cells containing a TGF β sensitive luciferase reporter gene. TGF- β 1 signaling from weakly DMCB-modified hydrogels was enhanced compared to the one obtained from highly DMCB-modified hydrogels, indicating a critical role for the DMCB content on the maintenance of the TGF- β 1 bioactivity.

The DMCB-modified hydrogels could be utilized to sequester TGF- β 1 for a long term signaling may be used as cell matrices for cartilage tissue engineering applications.

(26) Sealing the Bone-Implant Interface in Total Hip Arthroplasty Using Bone Marrow Stromal Cells (BMSCs)

Kalia P., Oussedik S., Konan S., Dodd M., Coathup M.J., Blunn G.W.

Centre for Biomedical Engineering, Institute of Orthopaedics, University College London, United Kingdom

Introduction: Aseptic loosening at the bone-implant interface of THA acetabular components is a significant cause of implant failure. Improving the fixation between the bone and implant may prevent loosening. The hypothesis of this study was spraying autologous BMSCs in fibrin glue onto the surface of HA-coated acetabular components, which would increase bone formation around the implant and improve bone-implant contact.

Materials and Methods: Goat BMSCs were isolated from bone marrow and expanded *in vitro*. BMSCs were suspended in thrombin pre-operatively. The acetabular shell was then coated with fibrin glue (+/- BMSCs) pre-implantation ($n = 6$). Walking and ground reaction forces were assessed pre-operatively, and 6 and 12 weeks post-operatively. After 12 weeks, the acetabulae were retrieved, and processed for histology.

Results: Overall new bone growth in the BMSC group $16.56\% \pm 8.97\%$ and $54.22\pm$ was 30% greater than the control group (71.42 respectively, $p = 0.58$). Bone-implant contact was significantly improved in the $8.32\% \pm 4.64\%$, in contrast to the control group ($13.71 \pm$ BMSC group ($20.03 p = 0.027$). There was no significant difference in loading between groups at both 6 and 12 weeks.

Discussion and Conclusions: Overall bone growth was greater when cups were treated with BMSCs, and bone-implant contact was also improved. A larger bony seal in contact with the implant may decrease the likelihood of aseptic loosening of THAs, and improve their longevity.

(27) Biodegradable Smart Cryogels as Bone Tissue Engineering Scaffolds

Bolgen N.¹, Aguilar M.R.², Fernandez M.³, San Roman J.², Pişkin E.¹

¹Hacettepe University, Chemical Engineering Department and Bioengineering Division, 06800, Beytepe, Ankara, Turkey

²Institute of Polymer Science and Technology (CSIC), Juan de la Cierva, 3. 28006 Madrid, Spain

³Facultad de Farmacia, Universidad Complutense de Madrid (UCM). Ciudad Universitaria s/n, 28040 Madrid, Spain

Biodegradable thermoresponsive cryogels were prepared by free radical polymerization of N-isopropylacrylamide monomer and a macromer composed of hydrolytically degradable oligolactate-2-hydroxyethyl methacrylate and dextran by cryogelation protocol. They were characterized by several techniques, i.e., FTIR, ¹H-NMR and SEM. Thermoresponsive behaviour of the cryogels with different chemical compositions was determined by swelling experiments and imaging NMR. These novel smart cryogels exhibited “volumetric phase change temperatures, VPCT” between 20°–37°C depending on the composition. The composition of cryogels and the medium temperature were found key parameters affecting *in vitro* degradation rate and profile. Two forms of simvastatin, hydrophobic and hydrophilic, were loaded into the cryogels following two different protocols: (I) embedding the drug after cryogelation, and (II) incorporating simvastatin during cryogelation.

FTIR and ¹H-NMR confirmed the drug loading. SEM micrographs demonstrated that hydrophobic simvastatin molecules were deposited on the walls of the pores of the cryogels, while hydrophilic simvastatin molecules were distributed within the solid polymer phase. *In vitro* release of simvastatin from the cryogels was investigated for possible application as Simvastatin delivering scaffolds for regeneration of bone defects. Different release profiles were observed in the *in vitro* release experiments, which were varied significantly depending on the type of simvastatin, the composition of the cryogel and the medium temperature.

(28) BioDynamic Stimulation of Orthopaedic and Vascular Biomaterials

Williams S., Lu J., Mejia L.

Bose Corporation, ElectroForce Systems Group, Eden Prairie, MN, United States of America

The objective of this work was to employ the novel design of the BioDynamic testing platform to evaluate the mechanical properties of hydrogels and other biomaterials. The testing platform allows for continuous stimulation and characterization (viscoelastic properties, strength, creep and stress relaxation) within a physiological environment (nutrient flow, pressure loading, pH, dissolved oxygen, and temperature) in a cell culture incubator.

The dynamic mechanical properties of hydrogels were evaluated with our unique computer-controlled moving magnet linear motor that provides load, displacement, strain or pressure profiles. In very low force applications, the peak-to-peak loading on the hydrogel was 2 mN with a peak-to-peak displacement of 28 microns. Upon completion of data acquisition using dynamic mechanical analysis, the software calculated the modulus and tan delta, which appeared to exhibit resonance between 20 and 100 Hz.

Vascular graft distension with increasing pressure was evaluated in a BioDynamic instrument using a laser micrometer. The graft material used (Gel-Del Technologies, Inc., St. Paul, MN) is composed of proteins and polymers fabricated to mimic the viscoelastic properties of native blood vessels. The diameter response followed the pressure changes very closely throughout the test. After each loading cycle, diameter did not return to its initial value within the test's time frame, indicating potential creep behavior.

Preliminary results with hydrogel discs and vascular grafts show that the BioDynamic test instrument is a powerful tool for the integration of biochemical and mechanical stimulation and characterization in one system.

(29) Biologically Active Wound Dressings Derived from Human Hair

Whitener W., Mesen T., Howse S., Nanney L., Molnar J., Van Dyke M.

Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, United States of America

Department of Plastic Surgery, Vanderbilt University School of Medicine, Nashville, Tennessee, United States of America

Hair follicle morphogenesis is a highly regenerative process that is mediated by a host of regulatory and matrix molecules. These growth factors would most certainly be extracted along with the keratins from end-cut human hair and impart biological activity to human hair keratin (HHK) extracts. In this study, we evaluated the ability of HHK extracts in mediating an improved wound healing response.

HHKs were derived using oxidation and reduction reactions to break down the tertiary structure of the cortex and extract soluble proteins. Immune competent mice were de-haired and a chemical burn induced between the shoulders using phenol. Wounds were treated after 20 minutes with a keratin hydrogel and dressings were changed every three days for up to 10 days. A series of deep partial thickness burns were also produced in pigs along the dorsal midline using a heated brass block. Wounds were treated every three days for up to 27 days. In a parallel series of cell culture assays, cell proliferation using keratinocytes and fibroblasts showed statistically significant increases in the keratose treated groups. In addition, wound healing studies in both mice and pigs demonstrated that the keratin treatments suppressed wound growth and accelerating healing compared to control groups.

Keratin biomaterials derived from human hair mediated the growth behavior of skin component cells. In cell culture experiments, certain types of keratins were mitogenic toward fibroblasts and keratinocytes. Keratin-based hydrogels were shown to be capable of passivating chemical and thermal burns in a mouse and pig model, respectively.

(30) Biomimetic Collagen-Hydroxyapatite Composite Scaffolds for Osteo and Chondrogenic Conduction of STRO-1 Immunoselected Human Bone Marrow Stromal Cells

Dawson J.I., Wahl D.A., Czernuszka J.T., Oreffo R.O.C.

Bone and Joint Research Group, University of Southampton, United Kingdom

Collagen's role in the temporal cascade of events leading to new bone from progenitors suggests it as a strong candidate material for tissue engineering scaffolds.

In an iterative approach, versatile, biomimetic type I collagen matrices of defined porosity, incorporating carbonate substituted hydroxyapatite (HA) crystals, were tailored and assessed for osteo- and chondrogenic conduction of STRO-1 immunoselected human bone marrow stromal cells (HBMSCs).

STRO-1 cells, dynamically seeded onto a collagen-HA composite (135 micron mean pore size) or pure collagen scaffold (64 micron mean pore size), were cultured in basal, osteogenic, or chondrogenic conditions for 21/28 days in comparison with a micromass chondrogenic model and calcium phosphate scaffold osteogenic model. Cell response was assessed by micro CT, histochemistry (Alcian blue/Sirius red, alkaline phosphatase {ALP}) and expression of osteo and chondrogenic markers by immunohistochemistry and RT-PCR.

Osteogenic differentiation on collagen-HA scaffolds was indicated by extensive ALP activity. Histology and micro-CT established cell penetration and new matrix synthesis with localised areas immunologically positive for osteocalcin. Chondrogenesis was evident in pellet and collagen systems by an abundance of sox-

9 expressing chondrocytes embedded in a proteoglycan and collagen II rich ECM. By RT-PCR, collagen and pellet cultures expressed Collagen II, IX and XI, Aggrecan, Osteopontin and Osteonectin and were negative for Collagen I and Osteocalcin.

Tissue specific gene expression and corresponding matrix synthesis indicate biomimetic collagen matrices to be highly conducive for the growth and differentiation of HBMSCs. These results demonstrate the potential of this platform for understanding the ECM cues necessary for osteo and chondrogenesis.

(31) Bioreactive Collagen-Hyaluronan Spiral Layers as Novel Sealing Collars for Tubular Implants

Foroughi F.¹, Anandagoda N.¹, Barker S.², Brown R.A.¹

¹University College London, Tissue Repair and Engineering Centre, Institute of Orthopaedics, London HA7 4LP, United Kingdom

²Evexar Medical Devices, 27 Fitzroy Square, London, United Kingdom

The fabrication and properties of a collagen-hyaluronan (C-H) over polyester tubular implants are described in which the controlled swelling of the C-H layer provides a novel approach to improve graft-host sealing without leakage for arterial and urethral repair. In vascular or urothelial implants tight seals between the implants and the irregular or diseased vessel lumen can be a major problem. We have previously characterised C-H sandwich layers to engineer localised swelling and adapted it here as a sealing cuff. Two types of C-H sheet layering were compared with hyaluronan either a) as a continuous sheet or b) as a depot on one side of the collagen sheet. The collagen-hyaluronan sandwich was wrapped around a polyester graft and supporting mandrel, collagen outermost, and freeze dried in place. Expansion of the constructs was assessed by 5 minutes swelling in PBS or water. In PBS, cuff swelling increased diameter by 10% over (collagen only) controls. Expansion in water was 28.2% and 30.1% for type a) and b) formats respectively. Geometrical expansions of 3 mm or more were generated to force soft collagen sponge into cavities or irregularities in the recipient vessel. The haemostatic nature of the collagen sponge means that clotting would also stabilise the seal (seen in scanning electron microscopy of construct), further preventing endoleakage.

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(32) Bioreactor Protocols for Improved Cellular Penetration of Decellularized Scaffolds

Yazdani S.K.^{1,2}, Jarajapu Y.P.R.², Machinga M.^{1,2}, Watts B.², Van Dyke M.E.², Christ G.J.²

¹Wake Forest University, Department of Biomedical Engineering, Winston-Salem, NC, United States of America

²Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, United States of America

Naturally derived biomaterials (i.e. decellularized tissue scaffolds) are favorable for cell survival due to their native architecture and

matrix compositions. However, cellular penetration and impregnation has proven challenging. This difficulty is largely related to the insufficient porosity of decellularized native tissue. To overcome this barrier we evaluated different seeding techniques and seeding environments, as well as the impact of altered cyclic strain during bioreactor conditioning.

Primary cultures of rat aortic vascular smooth muscle cells (VSMCs) were harvested and statically seeded (50 million cells/ml) on adventitia-denuded acellular porcine carotid arterial segments, followed by cyclic strain within a bioreactor system at a pulse rate of 60 beats/min and a pressure range of 80–120 mmHg in a bioreactor system for 2 weeks. In 10% FCS the VSMCs proliferated and aligned in multiple layers with little or no penetration into the scaffold. Reduction of the serum concentration to 1% during the second week of cyclic preconditioning favored VSMC penetration into the medial layer of the scaffold. Increased cyclic strain (i.e., 100–200 mmHg) did not further increase VSMC scaffold penetration.

These experiments are consistent with the supposition that high serum levels (i.e., 10% FCS) may be favorable during the initial cell attachment and proliferation period, but that prolonged exposure to high serum may maintain the VSMCs in a synthetic state, preventing effective cellular migration and penetration into the medial smooth muscle layer. Further investigation is required to identify critical growth factors responsible for this effect.

(33) Biphasic Scaffold for Tissue Engineering of Osteochondral Implants

Gelinsky M., Bernstein P., Eckert M.

Institute of Materials Science, Max-Bergmann-Centre of Biomaterials, Dresden, Germany

For the regenerative therapy of osteochondral defects—deep lesions of the articular cartilage in which the underlying bone tissue is also affected—special implant materials and scaffolds are needed. In this study, a new approach is presented, which is biphasic, but with monolithic scaffold material with tube-like pores. It consists of a mineralised layer for filling in the bony part of the defect and a non-mineralised layer for the chondral part. Due to the preparation method, both layers are fused together to give a unified whole without the need for any artificial joining. The resulting material, based on calcium alginate gels and hydroxyapatite, seems to provide a suitable scaffold for cultivating chondrocytes and osteoblasts and therefore can act as matrices for tissue engineering of osteochondral grafts. Stiffness and elasticity of the scaffolds were determined in uniaxial compression tests under wet conditions and seem to be favourable in therapy of osteochondral defects. For cell culture experiments, chondrocyte-alginate constructs were fabricated by adding the cells (primary chondrocytes, mini pig) to the non-mineralised alginate solvent before ionotropic gelation was initiated. The cell-loaded alginate constructs are cultured in a newly developed bioreactor combining an adjustable perfusion system with a mechanical stimulation jig. Mechanical stimulation as well as varying contents of hyaluronic acid in the chondral part support the maintenance of phenotype, which can be shown through immunohistology. Further studies will include co-culture of primary osteoblasts and

chondrocytes in the perfusion system and mechanical characterization of the cultured cell-alginate construct.

(34) Bone Angiogenesis Step One: Guided Cellular Infiltration

Keeney M., Damodaran G., Collin E., Pandit A.

National Centre for Biomedical Engineering and Science, National University of Ireland, Galway, Ireland

An optimal collagen/calcium phosphate (Coll/CaP) scaffold has yet to be constructed. In an idealised scaffold design, tethered biomolecules will aid cellular infiltration and promote neovascular network. It is hypothesised that the precursor for cellular infiltration is the existence of a structural micro-network. The aim of this study was to evaluate human osteoblast response to the scaffold architecture by assessing cellular proliferation, infiltration, morphology and gene expression. Coll/CaP scaffolds were created under physiological conditions [1] with 300 μ m diameter channels passing through the composite. Pore architecture was controlled and confirmed by micro-CT analysis. A range of pore distributions were chosen and seeded with 40,000 human SAOS2 cells ($n=3$). Static cultures were maintained for 21 days to monitor cellular response. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed for cellular RNA expression of osteocalcin as a biomarker for osteoblasts; alkaline phosphatase and collagen type I for matrix synthesis; and osteopontin for the ability to up-regulate endothelial cell migration induced by VEGF. [2] Fluorescent staining showed cellular adherence and migration into the pores while histological sections realised the extent of infiltration. By altering porosity and pore distribution, cell numbers, hence proliferation rates could be controlled. Gene expression indicated cellular phenotype was maintained. In conclusion Coll/CaP scaffolds have been designed and fabricated with a micro-network that successfully guides osteoblast infiltration and maintains cellular phenotype.

References

1. Bradt et al. (1999) J. Chem. Mater., 11, pp. 2694–2701.
2. Shijubo et al. (2000) Crit. Rev. Oncog., 11, 2, pp. 35–46.

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(35) Bone Marrow Progenitors from Animals with Chronic Kidney Disease Lack Capacity of *In Vitro* Proliferation

Kaznica A.¹, Drewa T.^{1,2}, Joachimiak R.¹, Flisinski M.³, Brymora A.³, Manitus J.³

¹Department of Tissue Engineering, Chair of Medical Biology, Medical College, Nicolaus Copernicus University, Bydgoszcz, Poland

²Chair and Department of Urology, Medical College, Nicolaus Copernicus University, Bydgoszcz, Poland

³Department of Nephrology, Hypertension and Internal Medicine, Medical College, Nicolaus Copernicus University, Bydgoszcz, Poland

Background: Interesting way to regenerate kidney is an autologous bone marrow transplantation. The aim of this study was to check if chronic kidney disease influenced bone marrow progenitors.

Methods: 4 Wistar rats underwent right nephrectomy (chronic kidney disease 1/2, CKD1/2). Right kidney and approximately 1/3 of the left kidney cortex were removed in 3 rats (CKD5/6). 4 animals in control were left intact. Serum creatinine, blood urea nitrogen (BUN) and haptoglobin levels were measured. Cells obtained from femurs were separated using CD34 Micro-Beads magnetic isolation kit and counted with trypan blue test. Number of isolated cells were presented as means with standard deviation. Means were compared using Student *t*-test. CD34(+) and CD34(–) cells were cultivated and observed until 6th passage.

Results: Serum creatinine levels were 0.83 ± 0.27 , 0.74 ± 0.07 and 1.22 ± 0.24 mg/dl in controls, CKD1/2 and CKD5/6, respectively ($p < 0.001$). BUN levels were 28.07 ± 4.00 , 48 ± 3.61 and 63.88 ± 17.85 mg/dl in controls, CKD1/2 and CKD5/6, respectively ($p < 0.001$). Haptoglobin levels were 1.68 ± 0.63 , 1.63 ± 0.62 and 0.70 ± 0.44 in CKD1/2, CKD5/6 and controls respectively ($p < 0.001$). No differences were found in CD34(+) and CD34(–) cell number after isolation in all animals. Cells in cultures established from control animals resembled normal fibroblast-like morphology of mesenchymal stem cells during 3 months. It was impossible to establish CD34(+) primary cultures. CD34(–) cultures were established from CKD rats but this cultures failed before 1st passage.

Conclusions: Bone marrow cells from CKD rats have no capacity for *in vitro* proliferation. We speculate that bone marrow cells from CKD patients cannot be used for autologous cell transplantation.

(36) Bone Morphogenetic Protein Delivery by Mesenchymal Stem Cells after BMP-2 Gene Electrotransfer

Ferreira E.¹, Potier E.¹, Vaudin P.², Logeart-Avramoglou D.¹, Mir L.L.³, Petite H.¹

¹Laboratoire de Recherches Orthopédiques, B2OA, UMR CNRS 7052, Paris, France

²Microenvironnement de l'Hématopoïèse et Cellules Souches, INSERM, EA3855, Tours, France

³Vectorologie et Transfert de Gènes, UMR 8121, CNRS, Villejuif, France

Although bone morphogenetic proteins (BMPs) are known to induce bone formation, their release in function of the biological context is a crucial purpose for bone tissue repair. Modulation of BMP release could be achieved by cells that overexpress a BMP transgene. We hereby hypothesize that: (i) the type of the promoter driving BMP gene may influence its expression, (ii) it is possible to modulate BMP secretion by an appropriate choice of the promoter.

Mesenchymal stem cells (MSCs) were electrotransfected with plasmids in which lacZ or BMP-2 gene was inserted under the control of different promoters. Beta-galactosidase and osteoblastic marker expression were monitored.

Beta-gal activity was clearly dependant on the promoter driving lacZ gene expression. Surprisingly, CMV promoter was less efficient than GAPDH or β -actin which led to a strong protein expression in undifferentiated MSCs. Osteocalcin promoter (pOG2) had a predictable weak activity as osteocalcin expression is expected at a later stage of MSC osteoblastic differentiation.

ALP expression (a marker of osteoblastic differentiation) after BMP-2 transfection was clearly dependent on the promoter fused to BMP-2 gene. For example at day 9, MSCs transfected with pGAPDH and peIF4A1/hBMP-2 showed maximal ALP staining and bone nodules whereas the ones transfected with pOG2/BMP-2 exhibited maximal ALP expression at day 18. Mineralized nodules were observed as soon as day 21 in MSCs transfected with peIF4A1/hBMP-2.

This study demonstrates that: (i) BMP-2 secretion by MSCs may be modulated by the promoter driving the transfected BMP-2 gene, (ii) promoters such as peIF4A1 or pGAPDH are more efficient than the CMV promoter.

(37) Bone Tissue Engineering in a Rat Cranial Critical Size Defect with Embryonic Stem Cells

Both S.K., Fernandes H.A.M., Blitterswijk C.A., De Boer J.

Institute for Biomedical Technology, Department of Tissue Regeneration, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands

In previous studies we demonstrated for the first time that mouse embryonic stem cells (mESCs) have the capacity of *in vivo* bone formation after *in vitro* being induced into the cartilage lineage. This process of bone formation is also known as endochondral bone formation. Because previous *in vivo* studies were performed in an ectopic model, we chose a rat critical size cranial defect as an orthotopic model to study the endochondral process by mESCs.

MESCs were seeded onto 8 mm calcium phosphate disks and induced into the cartilage lineage. Tissue engineered (TE) disks and sham disks, without cells, were implanted in the cranium of immune-deficient rats. As a control for ectopic bone formation TE particles were implanted in subcutaneous pockets. After 28 days *in vivo*, bone formation was observed in all TE cranial samples as well as in the ectopic controls. Several cranial disks displayed extensive bone formation. Since there was also bone in growth from the cranium into the outside of the sham controls, we compared the middle part of the sham implant versus the TE implant by histomorphometry. The amount of bone formed in the middle of the TE implants was significantly higher compared to the bone formed in the middle of the sham implants, where actually almost no bone formation was observed.

We have succeeded in making bone from mESCs in an orthotopic model for the first time, thereby showing that endochondral bone formation is a promising approach for *in vivo* bone formation by embryonic stem cells.

(38) Bone Tissue Engineering in the Oral Cavity using Synthetic Scaffolds and rhBMP-2

Schuckert K.-H., Jopp S.

Institute Indente, Ellernstraße 23, D-30175 Hannover, Germany

Tissue engineering particularly requires the use of synthetic and genetically produced substances. Against this background bone reconstruction within the oral cavity was done with TCP (tricalcium phosphate), PCL (poly-caprolactone) and rhBMP-2. ACS (bovine collagen), belonging to the medical kit of the BMPs, was not used as it is a xenogenic material. In addition PRP was employed.

The new possibility of Bone tissue engineering should be demonstrated exemplarily with some clinical cases to work out the advantages over the transplantation of autogenous bone being still considered as "golden standard" particularly with regard to large bony defects. As there is no bone removal, during this new method of bone reconstruction no secondary diseases appear which are otherwise noticed in many cases of bone reconstruction.

The results of *de novo* grown bone will be proved by X-rays pre- and postoperative and histological pictures. The quality of the new bone should be demonstrated as similar to the surrounding bone of the patient.

(39) Cardiac and Mesenchymal Stem Cell Growth and Selective Differentiation on Three Dimensional Bioerodable Scaffolds

Forte G., Carotenuto F., Vozzi G., Cossa P., Fiaccavento R., Minieri M., Pagliari F., Pagliari S., Romano R., Ahluwalia A., Traversa E., Di Nardo P.

University of Rome "Tor Vergata", Laboratory of Cellular and Molecular Cardiology, via della Ricerca Scientifica snc, 00133, Roma, Italy

The role of adult stem cells in cardiac homeostasis and repair has been recently addressed and the possibility that resident or circulating progenitors could be either activated or transplanted into injured hearts has raised new perspectives for the treatment of severe diseases. However, a few protocols based on the injection of cells into the injured site demonstrated the complexity of a cell-based approach to cardiac repair, suggesting the adoption of tissue engineering concepts to setup more suitable protocols. In the present study, a comparison between differentiative capabilities of mesenchymal (MSCs) vs. cardiac stem cells (CSCs) has been performed in bidimensional and tridimensional culture conditions. While both cell types showed multipotential capacity, the two subpopulations were clearly distinguishable on the basis of antigen expression and functional properties. When tested for their ability to grow on bioerodible polymeric scaffolds of poly-lactic acid (PLLA), poly-lactic-co-glycolide (PLGA) and poly-caprolactone (PCL) produced by pressure assisted microsyringe (PAM), both cell types expressed functional cell-to-cell and cell-to-matrix adhesion proteins. Moreover, they displayed different adhesive and proliferative behaviours while preserving their multipotential capacity on 2D polymers. Interestingly, when grown on 3D scaffolds with controlled tridimensional geometry, CSCs acquired mature cardiac phenotype and formed a quasi-tissue on PLLA polymeric matrix, while MSCs preserved their undifferentiated phenotype while growing in 3D culture conditions.

(40) Cartilage Regeneration with Bioactive, Cell-Free Tissue Engineered Implants

Endres M.^{1,2}, Neumann K.², Ringe J.¹, Erggelet C.³, Sittlinger M.¹, Kaps C.²

¹Charité-University Medicine Berlin, Dept. of Rheumatology, Tissue Engineering Laboratory and Berlin-Brandenburg Center for Regenerative Therapies, Tucholskystr. 2, 10117 Berlin, Germany
²TransTissue Technologies GmbH, Tucholskystr. 2, 10117 Berlin, Germany

³Department of Traumatology and Orthopaedic Surgery, University of Freiburg, Hugstetter Str. 55, 79106 Freiburg, Germany

Objective: The third generation of tissue engineering is characterized by the absence of *in vitro* expanded autologous cells but by the use of active substances, which support the self-healing capacity of the body. The guidance of progenitor cells (e.g. MSCs) towards the defect and subsequent formation of repair tissue opens new possibilities in the field of tissue repair. The objective was to recruit MSCs by chemoattractants, differentiate them with chondroinductors *in vitro* and verify the results in a sheep model in a cell-free approach.

Methods: Recruitment of human MSCs was tested using various chemokines, synovial fluid (SF) and serum in a modified boyden chamber assay. Chondrogenic differentiation was assessed by histological analysis and gene expression profiling after stimulating MSCs with hyaluronan and SF in high-density cultures. Furthermore, a combination of chemoattractants, chondroinductors and polyglycolic acid (PGA) scaffolds together with microfracture was used to treat full thickness cartilage defects in sheep joints.

Results: Besides SDF1 and IL8, SF and serum resulted in a distinct migration of MSCs. Chondrogenic differentiation of MSCs upon stimulation with SF or hyaluronan was shown by the formation of a proteoglycan-rich tissue *in vitro* and the induction of typical chondrogenic marker genes like type II collagen and aggrecan. The implantation of PGA scaffolds in the sheep joint resulted in the formation of a cartilaginous repair tissue. Controls treated with microfracture only, showed no formation of a repair tissue.

Conclusion: The PGA scaffold combined with chemoattractants and chondroinductors is well suited for the treatment of cartilage defects after microfracture and improves cartilaginous repair tissue formation.

(41) Cell Cooperation between Osteoprogenitor and Endothelial Cells for Bone Tissue Engineering

Grellier M., Bareille R., Bourget C., Ferreira Tojais N., Granja P., Barbosa M., Amédée J.

INSERM U577, Laboratory of Biomaterials and Tissue Repair, University V. Segalen, Bordeaux, France
 INEB, Biomedical Engineering Institute, Porto University, Porto, Portugal

Bone is a dynamic tissue that constantly undergoes remodelling. This process requires specific interactions between osteogenic cells but also between osteogenic cells and vascular endothelium cells.

The purpose of this work is to study cell signalling required in the osteoendothelial cell communication before applying this strategy for bone tissue engineering using a three dimensional system as vehicle for these cell types.

Human osteoprogenitor cells (HOP) isolated from bone marrow were cultured up to 48 hours in direct contact with human umbilical vein endothelial cells (HUVECs). Isolated cultures were used as control. Regulation of gene expression involved in extracellular matrix (ECM) production (MMP-1, TIMP-1, VEGF, TGFbeta-1) was investigated. We used immunostaining to observe the beta-catenin localization in control or co-cultures and phosphorylation of Smad proteins, involved in TGFbeta-1 signalling pathway, was analysed by Western Blot.

When HOP and HUVECs are in direct contact, they form specific multicellular networks. Time lapse microscopy revealed that HUVECs are able to migrate along the HOP cells. We observed a modification of beta-catenin distribution through the cells when they are in co-culture: beta-catenin is localised to the membrane while it's still cytoplasmic in isolated cultures. The balance between MMP-1 and TIMP-1 expression is in favor of a production of ECM and both VEGF and TGFbeta-1 are upregulated in these co-cultures. However, Smad signalling pathway seems to be not involved. These results suggest that an ECM remodelling and cell migration occurred when endothelial and osteoblastic cells are in contact. A better knowledge of this cell to cell communication will be helpful for the development of a vascularized bone tissue for tissue engineering.

(42) Cell Culture on Nano-Vibrating Surface for Controlling Cell Function

Ito Y., Kimura T., Higami T., Fujisato T., Kato A., Masuzawa T., Kishida A.

Tokyo Medical and Dental University, Institute of Biomaterials and Bioengineering, Tokyo, Japan
 Sapporo Medical University, Sapporo, Japan
 Osaka Institute of Technology, Osaka, Japan
 Ibaraki University, Ibaraki, Japan

Introduction: Handling cell function is one of important factors for preparing functional cell groups. Recently, physical stress, such as hydrostatic pressure and shear stress, has been studied for controlling cell function. These systems are inspired by physiological stress. To examine the influence of non-physiological stress on cell function, we developed nano-vibration system. In this study, we report the effect of nano-vibration stimuli on cell function—cell adhesion, proliferation and differentiation.

Methods: To investigate the influence of nano-vibration on cell adhesion and proliferation, L929 cell and MEF were used as typical cells. In the differentiation experiment, PC12 cell was used. All cells were vibrated at 10kHz for 1 hour everyday for 4 days. Then, the alternation was studied by counting cell number, observation of cell shapes and gene expression analysis using real-time RT-PCR.

Results: In L929 cell, there was no effect of nano-vibration on adhesion and proliferation. On the other hand, MEF cell showed drastic

change in adhesion and proliferation by nano-vibration. PC12 cell was hardly differentiated without nerve growth factor (NGF) addition, irrespective of nano-vibration. On the other hand, with NGF, the cell differentiation was promoted by nano-vibration in early culture period. However, the level of integrin and neuritin gene expression was not different in both nano-vibration and static culture.

Conclusion: We found that nano-vibration was effective on cell adhesion, proliferation and differentiation. These findings may lead to novel cell function controlling systems for stem and progenitor cells.

(43) Cell Culture on RGD-Containing Hydrogels

Perlin L., Rimmer S., MacNeil S.

University of Sheffield, Department of Chemistry, Western Bank, Sheffield, S3 7HF, United Kingdom

RGD-containing hydrogels have been synthesised via a novel strategy where the peptide is protected during synthesis and then removed by the enzyme Glutathione-S-Transferase (GST). Arginine derivatives were protected with the 4-bromobenzyl sulphonyl (4-Bbs) groups and the enzyme deprotected this group with efficiencies of 83 and 26% for Boc and Fmoc derivatives respectively.

The peptide sequence GRGDS was synthesised coupled to methacrylic acid directly or via a hydrophobic alkyl chain. Cross-linked hydrogels of glycerol methacrylate (GMA) or butyl methacrylate (BMA) and ethylene glycol dimethacrylate (EGDMA) were synthesised as thin films on PET by UV-initiated polymerisation.

The peptide promoted cell adhesion and viability in glycerol methacrylate (GMA) hydrogels but not in butyl methacrylate hydrogels. The presence of the spacer arm was not required in order for the peptide to promote cell adhesion in these randomly cross-linked systems. In addition to the peptide being deprotected by GST the protecting groups were also removed by cells in contact with the polymer and by non-contacting cells. The passage number of human dermal fibroblasts cultured on the materials affects the ability of the peptide to promote cell adhesion with higher passage numbers being more affected by the peptide. The morphology and F-actin organisation of cells cultured on the peptide containing hydrogels were different to that of those cultured on TCP.

(44) Cell Seeding Process Monitored by the Turbidimetric Method: The Kinetics of Cell Adhesion on Solid Scaffolds

Doaga I.O., Savopol T., Neagu A., Kovács E.

Carol Davila Univ. Med. & Pharm., Dept. Biophysics & Cell Biotechnology, 8 Eroii Sanitari Blvd., 050474-Bucharest, Romania
Victor Babeş Univ. Med. & Pharm., Dept. Biophysics & Medical Informatics, 2-4 Piata Eftimie Murgu, 300041-Timişoara, Romania

Purpose: Our goal was to develop a simple method to investigate the dynamics of the seeding process, as it involves all the key factors of tissue formation: cells, matrix and the interactions between them.

Methods: The proposed experimental setup works as an optical density-meter able to provide real-time information on the velocity of the cell seeding process, expressed as number of cells attached to the scaffold function of time. The experimental seeding curves were analyzed in terms of a kinetic model based on Langmuir's adsorption theory extended with a preliminary, irreversible step. We applied this model to interpret the results of experiments carried out with cells either trypsinized, or EDTA-treated, keeping for each case the same initial cell concentration and matrix properties. 3T3 murine fibroblasts and collagen type I sponges of same weight were used.

Results: Analytical interpretation of the seeding curves demonstrates that the seeding process was in both cases a sequential 2-step process. Though, the kinetic constants of the first step were higher in case of the cells detached with EDTA, while the kinetic constants for the second step were similar.

Conclusion: The circular flow cell seeding process, as was depicted by our dynamic experiments, is a sequential process that depends on the initial presence of integrin at the cell surface and, even though integrin is present from the beginning, cells have to readapt their integrin configuration from the 2D substrate to the 3D one.

(45) Cell Shape Regulates the Proliferation of Neural Precursor Cells

Ge D.¹, Liu T.Q.¹, Cui Z.F.², Guan S.¹, Ma X.H.¹

¹Stem Cell and Tissue Engineering Lab of Dalian University of Technology, Dalian, Liaoning, China

²Oxford Centre for Tissue Engineering and Bioprocessing, Department of Engineering Science, Oxford University, Oxford OX1 3PJ, United Kingdom

Commitment of stem and progenitor cells is regulated by many cues such as soluble factors, extracellular matrices (ECM) and multiple signaling in the local tissue microenvironment. Here we demonstrate that the cell shape forming in different culture manner can regulate the proliferation of neural precursor cells (NPCs). NPCs were isolated from E14 embryonic rat hippocampus tissue. Cells at 30 DIV allowed to be immobilized, adhered and spread in three-dimensional (3D) type I collagen gels incorporating fibronectin (FN), be suspended in culture media as neurospheres and be adhered and grew into monolayer on the FN coated planar dishes. The media were all serum-free but containing basic fibroblast growth factor and epidermis growth factor. We estimated their proliferative activity using the WST-8 assay and BrdU incorporation assay. And we detected GTP bound cellular Rho using affinity-precipitation assay since literature studies have shown that cell shape may affect the activity of Rho family GTPases and they are critical to proliferation. Our results show that cells cultured in different manner present different shape: cells entrapped in 3D microenvironment are sufficiently adhered and spread spatially, while cells in suspended neurosphere are round and monolayer cells are flat, adhered to ECM. Proliferation of NPCs in 3D collagen gel is better than that in the other two culture manners. The high Rho activity corresponded to the rapid spreading of cells in collagen gel incorporating FN in the initial 180 min. All the results demonstrate that cell shape, Rho activity are important cues to the proliferation of NPCs.

(46) Cell Sheet Tissue Engineering for Clinical Treatment

Okano T.

Institute of Advanced Biomedical Engineering and Science Tokyo Women's Medical University 8-1 Kawada, Shinjuku, Tokyo, 162-8666, Japan

Ideal tissue construction *in vitro* and *in vivo* can be achieved by cell sheet engineering that has been established with three core technologies: First, noninvasive harvest of cultured cells as cell sheets can be achieved using previously developed temperature-responsive culture dishes. The surfaces are hydrophobic at 37°C, but become hydrophilic below 32°C. Therefore, various cell types adhere, spread, and proliferate on the surfaces similarly to on commercial tissue culture dishes. By temperature reduction, cells spontaneously detach from the surfaces and confluent cells are recovered as a single contiguous monolayer sheet with intact cell-cell junctions and deposited extracellular matrix. Second, the harvested viable cell sheets can be transferred to other surfaces, such as culture dishes or devices (2D cell sheet manipulation) because of the adhesive extracellular matrix proteins associated with the basal side of cell sheets. Thus, tissue regeneration with cell sheet engineering can be accomplished by transplantation of single cell sheets, as with skin, cornea and periodontal ligaments. Finally, recovered cell sheets can be layered to reconstruct complex stratified tissue architectures such as liver lobules, kidney glomeruli, and cardiac patches (3D cell sheet manipulation). For example, layered cardiomyocyte sheets harvested from temperature responsive dishes show synchronous pulsations and diffuse gap junction formation. When transplanted into the subcutaneous tissues of nude rats, spontaneous beatings could be macroscopically observed and maintained for over 1 year. We believe that these 2D and 3D cell manipulations using cell sheet tissue engineering will become new and revolutionary tools for tissue engineering.

(47) Cell Sustainability in a Dendrimerically Cross-Linked Collagen Scaffold

Tiong W.H.C., Damodaran G., Kelly J.K., Pandit A.

National Centre for Biomedical Engineering and Science, National University of Ireland, Galway, Ireland

Starburst dendrimer has been incorporated into many polymers in order to achieve novel functional materials (1). It is hypothesized that a PAMAM-incorporated, EDC cross-linked collagen scaffold will improve its functionality.

0.3% collagen scaffolds were fabricated, and cross-linked with 5 mM, 15 mM, and 25 mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) concentration in 5mM N-hydroxysuccinimide (NHS) and 50 mM 2-morpholinoethane sulfonic acid buffer at pH 5.0. PAMAM with weight to collagen volume ratio of 5:1 and 10:1 were also added for each EDC concentrations respectively and incubated overnight at 37°C, and freeze-dried. These scaffolds were characterised by collagenase degradation, FTIR spectra, Alamar Blue cell viability, and morphology with scanning electron microscopy (SEM) and fluorescence cell staining.

Collagenase degradation at 10 units/mg collagen showed significant decrease in degradation of the cross-linked collagen. FTIR

analysis showed increased extent of cross-linking of collagen with the addition of 5:1 dendrimer to collagen ratio. The amide A band arising from NH stretching at 3301.91 cm^{-1} was considerably influenced by the cross-linking. SEM images showed cross-linked collagen fibres formed a more uniform surface for cell attachment. This is supported by collagen gel cross-linked with 5 mM EDC/ 5 mM NHS at 5:1 dendrimer:collagen ratio which showed a significantly improved cell proliferation at both day 3 and 5. Further SEM images and fluorescence cell staining confirmed normal cell morphology. In conclusion, a dendrimeric collagen scaffold system has been developed which sustained cell viability and scaffold stability.

Reference

1. Chung et al. Langmuir 2004; 20:2966–9.

(48) Cell Viability and Morphology on Starburst Dendrimer-Crosslinked CEM ScaffoldsChan J.C.Y.^{1,2}, Burugapalli K.¹, Kelly J.L.², Pandit A.¹

¹National Centre for Biomedical Engineering and Science, National University of Ireland, University Road, Galway, Ireland

²Department of Plastic Surgery, University Hospital Galway, Newcastle Road, Galway, Ireland

Intact extracellular matrices have demonstrated potential in clinical applications. In order to improve the potential of these natural matrices further, exogenous bioactive molecules or growth factors could be tethered. In this study, cholecyst-derived extracellular matrix was crosslinked with G1 Starburst (PAMAM) dendrimer. We hypothesised that the multi-amine terminated surface of PAMAM dendrimer incorporated in an extracellular matrix would provide additional grafting site for the delivery of these substrates. Cholecyst-derived extracellular matrix (CEM) was crosslinked using EDC/NHS (1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide/N-hydroxysuccinimide) (0.0066 mM, molar ratio 1:1 in 50 mM of MES buffer, pH 5.5). Varying amounts of PAMAM dendrimer (0.35, 0.7, 2.1 and 6.3 mM/mg) were added to the reactant solution. The resulting scaffolds were freeze dried. Fibroblasts were seeded on these scaffolds at a density of 20000 cells/scaffold with DMEM supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin and 1.25 mg/L amphotericin-B. Uncrosslinked and EDC/NHS-crosslinked scaffolds without PAMAM were used as control. Cell viability was evaluated using AlamarBlue assay at day 1, 3, 5 and 7. The morphology of cells on these scaffolds was examined using scanning electron microscopy (SEM) and fluorescent staining (DAPI and rhodamine phalloidin). Cells proliferated on all scaffolds. There was no significant difference between different treatment types at each time point. SEM and fluorescent staining showed normal fibroblasts morphology. Multiple processes extended from the cell body and attached on the CEM fibres. Areas of confluent cell growth were seen at day 7. It is concluded that crosslinking of CEM with Starburst dendrimer did not effect fibroblast viability.

(49) Cell Viability of Cultured Corneal Endothelial Cells for Tissue Engineering: The Effect of Cell Density

Gonzalez-Andrades M., Garzon I., Muñoz-Avila J.I., Alaminos M., Serrano D., Medialdea S., Campos A.

Department of Histology (Group of Tissue Engineering), University of Granada, Spain
Division of Ophthalmology, University Hospitals San Cecilio and Virgen de las Nieves, Granada, Spain

Introduction: Several scientists have tried to construct corneal equivalents by tissue engineering (1) and to determine corneal cell viability (2). However, the influence of cell density on the functionality of the corneal endothelial cells is unknown. In this work, we tried to determine the role of cell density on the ionic cell content in cultured corneal endothelial cells.

Materials and Methods: Endothelial cell cultures were established using laboratory rabbit corneas. Quantification of the ionic contents of Na, K, S, P, Ca, Mg and Cl in confluent and subconfluent endothelium was carried out by electron probe X-ray microanalysis using a Philips XL30 scanning electron microscope with an energy-dispersive detector EDAX. Confluent and subconfluent cells were analysed using the P/B ratio method with reference to standards of inorganic salts.

Results and Discussion: When compared to confluent cells, subconfluent corneal endothelial cells showed lower levels of Na and Cl and higher levels of K. No differences were found for S, P, Ca and Mg. Our data suggest that confluency might be a key factor inducing activation of membrane pumps and cell mechanisms involved in maintaining of the ionic equilibrium of the cornea. For that reason, confluent cells should be used in corneal constructs.

References

1. Alaminos et al. (2006). Construction of a complete rabbit cornea substitute using a fibrin-agarose scaffold. *Invest Ophthalmol Vis Sci*. 47:3311–3317.
2. Alaminos et al. (2007). Evaluation of the viability of cultured corneal endothelial cells by quantitative electron probe X-ray microanalysis. *J Cell Physiol*. 211:692–698.

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(50) Cell-Matrix Dynamics of Tissue Engineered Ligament Under Static and Perfusion Culture

Hillenmeyer E., Hunt J.A., Rhodes N.P.

UK Centre for Tissue Engineering, University of Liverpool, Clinical Engineering, Liverpool, L69 3GA, United Kingdom

Introduction: For materials to be used in load-bearing applications such as ligaments, the mechanical properties should be optimized in order to promote effective integration. Culturing constructs under perfused flow regimes increases proliferation and extracellular matrix production [1, 2]. However, it is poorly understood how to translate these to controlling mechanical properties.

Methods: 3D fibre scaffolds (5 mm diameter) of esterified hyaluronan (HYAFF) and poly-lactic acid (PLA) (Fidia Biopolymers) were cultured with and without human fibroblasts and incubated under static or flow perfusion (6 mm flow rate) conditions. Constructs were analyzed for cell metabolism using MTT assay. Collagen production was determined histologically (van Geissson

stain). The mechanical properties of constructs were tested by tensile testing (30 mm/min).

Results/Discussion: Control acellular HYAFF-PLA 3D scaffolds showed degradation of mechanical properties over time. Fibroblast seeded scaffolds cultured under static conditions showed a reduction in their mechanical properties compared to the acellular controls. Fibroblast seeded constructs cultured under flow perfusion conditions also demonstrated changes in their mechanical properties compared to the acellular controls and the static conditions; indicating that the cell-matrix interactions governing the mechanical properties can be modified and controlled by culture conditions.

References

1. Bancroft, G.N., et al., Technical Note: Design of a Flow Perfusion Bioreactor System for Bone Tissue-Engineering Applications. *Tissue Engineering*, 2003. 9(3): p. 549–554.
2. Rhodes, N.P., et al., Metabolic and histological analysis of mesenchymal stem cells grown in 3-D hyaluronan-based scaffolds. *Journal of Materials Science: Materials in Medicine*, 2004. 15(4): p. 391–395.

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(51) Cell-Seeded Microbeads as Carriers for Tissue Delivery

Imparato G., Urciuolo F., Palmiero C., Netti P.A.

Interdisciplinary Research Centre on Biomaterials (C.R.I.B.), University of Naples, Federico II and Institute for Composite and Biomedical Materials (I.M.C.B.), National Council Research, P.le Tecchio 80 80125, Naples, Italy

Tissue engineering hold enormous potential to regenerate tissue *in vitro* and *in vivo*, however conventional application using 3D scaffold has revealed several limitation to *in vitro* production of 3D functional tissue equivalent. To overcome this problem novel strategies have been proposed, which rely upon the realization of *in vitro* 2D tissue layer that can further assemble to obtain 3D more complex tissue. Okano [1] has recently shown that cell sheets put in contact adhered each other because of the presence of deposited extracellular matrix (ECM) realizing more complex and thicker tissue where the intercellular communication was maintained. Following this approach, we propose a novel strategy that aim at producing small pieces of tissue (μ tissue) that can be used as building block for more complex tissue.

To this aim we succeeded in expanding bovine fibroblasts on gelatine microcarrier spinner culture to support 3D μ tissue formation. By setting the process condition in order to encourage cell to microbeads adhesion rather than beads to beads aggregation, cells adhered and grown in and around the macroporous microbeads and synthesized ECM. That led to a deposition of a thin layer of dermal matrix components through the beads, generating a 3D μ tissue precursor able to grow in diameter until 4 days of spinner culture in dilute conditions.

We have found by keeping in contact the 3D μ tissue precursors that they were able to assemble creating a more complex and thicker tissue suggesting the possibility of using cell seeded microbeads as tissue delivery system for *in vitro* and *in vivo* applications.

Reference

1. "Cell sheet engineering: Recreating tissues without biodegradable scaffolds" J.Yanga, M. Yamatoa, C. Kohnoa, A. Nishimoto, H. Sekinea, F. Fukaib, T. Okano. *Biomaterials* 26 (2005).

(52) Cellular Delivery of DNA-Polymer Complex Encapsulating Inorganic Nanoparticles Prepared by Ultra High Pressurization

Kimra T., Okada M., Furuzono T., Yoshizawa H., Fujisato T., Kishida A.

Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo, 101-0062 Japan

Dept Bioengineering, National Cardiovascular Center Research Institute, Suita city, Osaka, Japan

Department of Material and Energy Science, Okayama University, Okayama City, Okayama, Japan

Department of Biomedical Engineering, Osaka Institute of Technology, Asahi-ku, Osaka, Japan

We have developed gene delivery system using DNA complex with non-ionic, water soluble polymers via hydrogen bond through ultra high pressurization (UHP) because the inter-, intra-molecular weak hydrogen bonding interaction was strengthened under high pressure condition. Previously, polyvinyl alcohol (PVA) was used as a model hydrogen bonding polymer, and the PVA/DNA complexes were formed by UHP treatment. Although the PVA/DNA complexes were up-taken by cells, a little enhancement of gene expression was observed using them. Therefore, in this study, to promote the endosomal escape of transferred DNA, we performed the development of PVA/DNA complexes encapsulating inorganic particle, which are dissolved under low pH condition in endosome vesicles and then the rupture of endosome is induced by osmotic shock, using UHP technology. Plasmid DNAs encoding luciferase gene or enhanced green fluorescent protein (EGFP) gene under CMV promoter were used. Nano-scaled inorganic particles having the average diameter of 50–200 nm were synthesized by modified micro-emulsion method. Nano-inorganic particles were dispersed ultrasonically in PVA solution and then mixed with DNA solution. Their mixtures were treated under 10,000 atmospheric pressures at 40°C for 10 min. By SEM observation, the irregular surface of PVA/DNA complexes including inorganic particles was observed, indicating the encapsulation of inorganic particles in PVA/DNA particle. The PVA/DNA complexes encapsulating inorganic particles showed a higher transfection activity. These results indicate the utility of the PVA/DNA complexes encapsulating inorganic particles prepared by UHP method for DNA delivery.

(53) Cellular Growth Under Cyclic Hydrostatic Pressure in Cells Cultured on an Acellular Scaffold in a Novel Bioreactor

Shaikh F.M.^{1,2}, Brien T.P.O.², Callanan A.², Kavanagh E.G.^{1,2}, Burke P.E.^{1,2}, Grace P.A.^{1,2}, Badylak S.F.³, McGloughlin T.M.²

¹Department of Surgery, Mid-Western Regional Hospital, Limerick, Ireland

²Centre for Applied Biomedical Engineering Research, University of Limerick, Limerick, Ireland

³Center for Pre-Clinical Tissue Engineering, McGowan Institute for Regenerative Medicine, Pittsburgh, Pennsylvania, United States of America

Introduction: Mechanical forces in the circulatory system are important regulators of cell behaviour in the vessel wall. Bioreactors developed for vascular tissue engineering mimic these physiological mechanical strains on the blood vessels. In contrast to extensive work on shear stress and cyclical strain, to date there has been only limited studies on the role of pressure on the behaviour of endothelial cells. Moreover the effects of pulsatile pressure on cells grown on a natural scaffold have not been examined. Our aim was to examine the effects of hydrostatic pressure on endothelial cell growth on an acellular natural scaffold.

Methods: Human Umbilical Vein Endothelial Cells (HUVEC) were grown on an acellular scaffold derived from porcine urinary bladder membrane (UBM) in a novel bioreactor. Seeded HUVEC UBM co-constructs were exposed to either atmospheric or pulsatile pressure (50/110 mmHg) inside the bioreactor for 24 hours. After the experiments cell and matrix morphology was examined using optical and scanning electron microscopy (SEM). Cell viability was assessed using live-dead immunofluorescence and cell count kit 8.

Results: Excellent cell growth and maintenance of matrix integrity were demonstrated by means of light and scanning electron microscopy under pressure conditions. Moreover endothelial cells showed uniform cellular alignment and formed a monolayer under physiological pressure conditions.

Conclusion: The results showed that UBM has a potential as a biocompatible natural acellular vascular scaffold capable of performing within physiologically realistic environments and our novel bioreactor is capable of providing a physiologically realistic environment for the vascular biological constructs.

(54) Cellular Synergy for 1 Step Cartilage Repair

Hendriks J., Schotel R., Van Blitterswijk C.A., Riesle J.

CellCoTec BV, Prof. Bronkhorstlaan 10D, 3723 MB Bilthoven, The Netherlands

University Twente, BMTI, 7500 AE Enschede, The Netherlands

Purpose: One of the current treatments for cartilage defects is based on expanded chondrocytes. Expansion of chondrocytes is, however, expensive, requires a complicated procedure including 2 surgeries, and hampers cellular quality as it results in cell dedifferentiation. To address these drawbacks, we hypothesized that primary chondrocytes and bone marrow cells synergize in cartilage formation. We further hypothesized that the required number of chondrocytes can be isolated from a small biopsy within surgery time. Together with an intra-operative source for bone marrow cells, a simple one surgery treatment for cartilage defects may be developed.

Objective: The aim of this study was i) to investigate if a combination of chondrocytes and bone marrow cells synergize in enhancing cartilage formation *in vitro* and *in vivo*, and, ii) to establish chondrocyte isolation within less than an hour.

Methods: For the first study, we combined primary chondrocytes and expanded bone marrow cells into micromass pellets or into polymer scaffolds, cultivated *in vitro* or implanted into nude mice, and analyzed for glycosaminoglycans (GAG) and collagen type II. For the second study, we dissected cartilage, enzymatically digested for various time periods, and analyzed for cell number and viability.

Results: *In vitro* and *in vivo* results showed that constructs comprising 20% primary chondrocytes and 80% bone marrow cells contain significantly more GAG compared to the expanded chondrocyte control, and a comparable amount of GAG compared to the 100% primary chondrocyte control. A substantial chondrocyte number could be isolated within 10–30 min.

Conclusion: Cellular synergy may provide the basis for a one surgery cartilage defect treatment.

(55) Channel Networked Collagen Scaffolds for Tissue Engineering

Liu C., Xia Z., Hulley P.A., Triffitt J.T., Williams D.J., Czernuszka J.T.

The Wolfson School of Mechanical & Manufacturing Engineering, Loughborough University, United Kingdom
Botnar Research Centre, University of Oxford, Headington, Oxford OX3 7LD, United Kingdom
Department of Materials, University of Oxford, Parks Road, Oxford OX1 3PH, United Kingdom

This paper reports the strategy for the fabrication of collagen-hydroxyapatite composite scaffolds using a 3D printing technique to achieve such a controlled architecture. The *in vitro* performance of the scaffold in terms of cells' viability, migration and proliferation behavior using human mesenchymal stem cells (hMSC) is reported. The purpose of this paper is to study the effect of micro-channels on the cell migration, proliferation and differentiation.

The collagen-hydroxyapatite composite scaffolds were fabricated by using an indirect 3D print technique. The process involves fabrication of a sacrificial negative mould, casting of the collagen/HA dispersion, removal of the negative mould and dehydration process. The micro-structure of the resultant scaffolds have been characterised by micro-CT, SEM and FIB examinations, associated mechanical properties were investigated by dynamic mechanical analysis (DMA). The cell viability/proliferation was evaluated *in vitro* using human mesenchymal stem cells (hMSCs).

It was demonstrated that the scaffold featured predefined internal microchannels and interconnected pores network. The matrix has a porosity of 92%, with pore sizes distributed in the range of 100 ~ 300 µm. The dynamic mechanical properties and biodegradation profile are adjustable by crosslinking treatment. The *in vitro* evaluation revealed that the micro-channels could be well preserved during cell culture of 8 weeks *in vitro*, the hMSC can migrate into the scaffold, preferably through the micro-channels to proliferate and differentiate. A combination of histological examination (include Alizarin red and Alcian blue, and ALP staining) revealed bone and cartilage-like tissue formed after 8 weeks culture.

(56) Characterisation of Bone Marrow and Cultured Human and Goat Mesenchymal Stromal Cells

Rozemuller H.¹, Prins H.¹, Staal J.M.¹, Bühring H.J.², Martens A.C.M.¹

¹Department Immunology, Utrecht Medical Center, Utrecht, The Netherlands

²Department of Internal Medicine, University Clinic of Tübingen, Tübingen, Germany

Our group is interested in human and goat bone forming capacity of mesenchymal stromal cells (MSC). In contrast to good bone production by goat MSC (30–40% of scaffolds filled with bone), human MSC only give rise to a minimal amount of bone (1–5%). Our hypothesis is that the potency of stromal cell population might be improved when the stem cells within the MSC population or the cells with a high proliferative capacity can be isolated, expanded, and seeded onto scaffolds. To improve the “stem cell quality” of seeded human MSC we focus on stem cell purification by flow sorting or magnetic cell sorting on the basis of specific antibodies (Abs). We tested the commercially available Abs Stro-1 and LNGFR, and a number of new Abs that recognize subpopulations of the LNGFR positive fraction of human bone marrow, which were developed by Dr. HJ Bühring. We have tested the Abs on 4 human BM samples, 3 human MSC cultures, and also on 2 goat BM samples, and 2 goat MSC cultures for cross reactivity (see table). Beside the NGFR and the Stro-1, 3 new Abs were positive on bone marrow (BM) cells from both species. FACSsorting of the Ab positive human BM cells demonstrated the enrichment of CFU-F. Cells are now expanded and the proliferative capacity, multipotency and the potential of *in vivo* bone formation of the selected cells are ongoing.

	huMSC	huBM	HuCFU-F	GMSC	GBM
(enrichm.)					
LNGFR	–	+	+ (26×)	–	+
Stro-1	+	+	not done	+	+
W3C4E1	+	+	+ (21×)	+	+
W8B2B10	+	+	+ (14×)	+	+
W4A5B5	+	+	+ (11×)	+	+

(57) Characterisation of Gel-Like Materials for Tissue Engineering

Lam J.K.W.L., Tomlins P.E.

National Physical Laboratory, Teddington, Middlesex, TW11 0LW, United Kingdom

Gels are used as drug release housings or as matrices for hosting cells for sensor applications or for regenerative medicine. Characterising these materials, in terms of structural features, such as the distribution of pore sizes or the degree of structural homogeneity, is challenging due to their high water content and fragility. Microscopic studies usually require drying of the sample that can be done

by, for example, freeze drying or progressive removal of water using ethanol/water mixtures. Regardless of the preparation method there is a very high risk of introducing artefacts which are very difficult to quantify.

An alternative approach to characterisation is to determine performance using molecular probes. This technique relies on measuring the permeability of the gel to a range of differently sized or charged molecules. A range of methods are available for making such measurements, these can be grouped into those that measure diffusivity within the gel and those that determine molecular transport across it. Examples of the former include FRAP (fluorescence recovery after photobleaching) and simple methods for tracking gravity induced permeation. Molecular transport across gels can, depending on their resilience, be studied using simple diffusion cells or electrochemical techniques.

In this paper we present a comparison of molecular probe diffusivity measurement within and across gels using a range of techniques and probes. The results demonstrate the importance of understanding the scope of the measurement method and its impact on repeatability and uncertainty.

(58) Characterisation of Human Fetal Progenitor Populations and Response to Osteogenic Growth Factors: A Model System for Mesenchymal Lineage Differentiation

Mirmalek-Sani S.H.¹, Tare R.S.¹, Hayes A.J.², Caterson B.², Hanley N.A.³, Houghton F.D.³, Oreffo R.O.C.¹

¹Bone and Joint Research Group, Developmental Origins of Health and Disease Division, University of Southampton, SO16 6YD, United Kingdom

²Cardiff School of Biosciences, Cardiff University, Cardiff, CF10 3US, United Kingdom

³Human Genetics Division, University of Southampton, SO16 6YD, United Kingdom

Mesenchymal populations derived from skeletal tissues provide a platform for cell-based tissue regeneration strategies. Cell populations from human fetal tissue may offer a unique comparative model system for differentiation studies. Cells were isolated from human fetal femur tissue at 8–11 weeks postconception and grown in basal culture conditions with an average population doubling time of 21.5 ± 1.0 hours. Flow cytometry analysis of day 12 basal cultures indicated the presence of osteoprogenitors (3–10%) and chondroprogenitors (4–11%) using the STRO-1 antibody and the novel 7D4 antibody (which recognises Versican proteoglycan chains) respectively. Expression of the STRO-1 antigen was observed in cultures after 21 days and as late as passage 3. Culture in osteogenic conditions (ascorbate/dexamethasone) significantly increased alkaline phosphatase (ALP) in a dose-dependent manner. However, addition of osteogenic factors BMP2, Pleiotrophin, Simvastatin or Vitamin D3 resulted in significantly decreased ALP activity in comparison to control cultures. Maintenance in chemically defined media without serum generated a unique undifferentiated cell population. Examination of fetal progenitor response under serum-free chemically defined media (CDM) conditions indicated comparable cell numbers to cultures grown in the presence of serum, however, cells exhibited significantly reduced ALP activity. Application of ascorbate/dexamethasone to CDM cultures resulted in increased alkaline phosphatase activity,

however rescue of phenotype could only be achieved in the presence of serum. Current studies are focused on elucidating the mechanisms underlying the differential fetal progenitor cell response and their potential for rescue studies and lineage modulation.

(59) Characterization of Schwann Cell According to Passage and Culture Medium for Neural Regeneration Using Tissue Engineering

Oh A.Y., Kim S.H., Kim S.M., Kim C.M., Rhee J.M., Kim M.S., Lee H.B., Khang G.

BK-21 polymer BIN Fusion Research team Chonbuk National University, Nanobiomaterials Lab, Korea Research Institute of Chemical Technology, Korea

Each year, approximately 11,000 Americans suffer from spinal cord injuries and annual cost of medical care has been estimated at about \$11 billion to treat spinal cord injuries. Several early studies observed that Schwann cells (SCs) have been accepted to play indispensable roles during neural development and regeneration. The aim of this study is to examine a phenotype of SCs according to passage and culture medium.

SCs were isolated by using modified Morrissey method from hind leg of rat. These cells were plated at 1.5×10^4 cells/cm² and cultured in Medium 1 (DMEM/F12, fetal bovine serum (FBS), penicillin streptomycin (PS), forskolin and bovine pituitary extract (BPE)), Medium 2 (DMEM/F12, FBS, PS) and Medium 3 (DMEM/F12, FBS, PS, forskolin), respectively. And then, we subcultured from passage 1 to 5.

The morphology of cells depending on passage was examined using Nikon inverted microscope. RT-PCR was conducted to confirm mRNA expression of S-100 and p75 for SC marker. Also, cultured cells from passage 1 to passage 5 were stained for anti S-100 and p75 by ABC method. We confirmed that SCs showed different morphology and phenotype according to passage and culture medium through morphology, RT-PCR and immunocytochemistry. Specially, the morphology of SCs was apparent in Medium 1 with passage 3. In conclusion, these characterizations of SCs would provide suitable condition for application of SCs in terms of spinal cord regeneration.

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(60) Characterizing Human Circulating Progenitor Cells on a Novel Biodegradable Elastomer

Allen J., Ameer G.A.

Northwestern University, 2145 Sheridan Rd., Evanston, IL 60208-3100, United States of America

Tissue engineered vascular grafts may one day provide a solution to many of the limitations associated with the use of synthetic vascular grafts. However, identifying a suitable cell source and polymer scaffold to fully re-create the properties of a native blood

vessel remains a challenge. In this work, we assess the feasibility of using endothelial progenitor cells (EPC) found in circulating blood to generate a functional endothelium on poly(1,8-octanediol-co-citrate) (POC), a biodegradable elastomeric polyester. EPCs were isolated from human blood and biochemically differentiated into endothelial-like cells (HE-like) *in vitro*. The differentiated cell phenotype and function were confirmed by positive staining for endothelial cell specific markers, von Willebrand factor, CD144 (Vascular Endothelial-cadherin), flk-1, and CD31 (PECAM-1). Additionally, the HE-like cells cultured on POC produce and secrete anti-thrombogenic factors nitric oxide (NO), prostacyclin (PGI₂), and tissue plasminogen activator (tPA). Further, a monolayer of HE-like cells cultured on POC shows a decrease in the rate of clot formation of recalcified plasma, as well as whole blood, *in vitro*. Collectively, these data suggest that a POC scaffold seeded with human endothelial-like cells could have a major impact on vascular tissue engineering.

(61) Chemokine Receptor Expression and Chemotaxis in Human Dermal Fibroblasts

Kroeze K.L.¹, Bruijnzeel D.P.¹, Scheper R.J.², Gibbs S.¹

VUMC, Department ¹Dermatology & ²Pathology, Amsterdam, The Netherlands

Background: During wound healing chemokines and their receptors play an important role in site-directed migration of dermal fibroblasts into the wound. This is required for tissue regeneration. In the future it may be possible to derive a 'chemokine cocktail' which will stimulate growth and targeted migration of dermal fibroblasts into a burn-wound (2nd grade) or a dermal substitute applied to a 3rd grade burn-wound. The aim of this research is to study the chemokine-receptor expression and chemotaxis of human dermal fibroblasts.

Methods: Expression of CCR1, 2, 3, 4, 5, 8, 10 and CXCR1 and 2 was characterized on freshly isolated and cultured human dermal fibroblasts by means of flow cytometry and RT-PCR. The effects of the corresponding chemokine-ligands on dermal fibroblast migration (scratch assay), on chemotaxis (microchemotaxis chamber technique) and on the proliferation (BrdU assay) were evaluated.

Results: CCR 3, 4, 10 and CXCR1 and 2 are constitutively expressed in freshly isolated and cultured dermal fibroblasts. Ligands for these receptors can be divided into 4 groups, ranging from weak to strong migration-inducing capacity. Only ligands corresponding to CXCR1 and CXCR2 enhance the proliferation of the dermal fibroblasts.

Conclusion: Migration and proliferation of dermal fibroblasts are differentially regulated by chemokines. More research on differential effect of chemokines on skin residential cells should finally result in a 'chemokine cocktail' suitable for clinical use, aimed at optimal tissue regeneration in difficult to heal wounds.

(62) Chitosan Based Delivery Systems for Injectable Tissue Engineered Bone: Human Mesenchymal Stem Cell Survival, Proliferation and Osteogenic Differentiation

Ahmadi R., De Bruijn J.D.

School of Engineering and Materials Science, Queen Mary University of London, Mile End Road, London, E1 4NS, United Kingdom

Chitosan is a natural biopolymer which combined with glycerol phosphate (GP) can undergo gelation at 37°C (1). Addition of hydroxyethyl cellulose (HEC) ensures that the biomaterial scaffolds remain homogeneously distributed within the gel. With the increasing popularity of non-invasive surgery, injectable thermosensitive chitosan is particularly promising as cell/scaffold delivery vehicle for bone regeneration. The aim of this study is to evaluate the proliferation of human mesenchymal stem cells (hMSCs) exposed to different gel compositions as well as to examine hMSCs survival and osteogenic differentiation within chitosan-GP-HEC for the purpose of creating injectable formulations of tissue engineered bone.

Metabolic activity of hMSC exposed to 1.5%(w/v) chitosan-15%(w/v) GP mixed with 0%, 0.18%, 0.36%, and 0.72%(w/v) HEC was assessed over a 7 day period using Alamar blue assay. hMSCs (30,000 cells/cm²) were cultured on 212–300 µm biphasic calcium phosphate (BCP) microparticles for 7 days after which chitosan-GP-HEC was added such that the final BCP/gel ratio was 20% (w/v). Live/dead staining using Calcein AM and Ethidium homodimer along with evaluation of alkaline phosphatase activity (ALP) and protein content was done before and after gel addition over a 14 day period.

Low HEC concentrations of 0.18% (w/v) showed the highest cell growth compared to other concentrations. hMSCs attached to BCP particles exposed to chitosan-GP-HEC, proliferated, and remained viable for at least 7 days. The ALP activity of hMSCs normalized to total protein content in control and chitosan-GP-HEC surrounded groups reached a peak at day 10.

Reference

1. Chenite *et al.* (2000) Novel injectable neutral solutions of chitosan form biodegradable gels in situ. *Biomaterials* 21:2155–2161.

(63) Chondrocytes Express Primary Cilia Decorated with Connexin 43 Hemichannels Providing a Pathway for Purinergic Mechanotransduction

Knight M.M.¹, McGlashan S.R.², Jensen C.G.², Poole C.A.²

¹Faculty of Medical and Health Sciences, University of Auckland, Private Bag 92019, New Zealand

²School of Engineering and Material Science, Queen Mary University of London, Mile End Rd., London, E1 4NS, United Kingdom

Mechanical loading is essential for the health and homeostasis of articular cartilage and may be utilised within a tissue engineering context to promote the production of a functional repair tissue. In previous studies we have demonstrated that compression of chondrocytes activates an autocrine/paracrine Ca²⁺ signalling pathway, mediated by the release of ATP, which triggers an up-regulation of proteoglycan synthesis. However, the mechanism of ATP release is unclear.

The present study used immunofluorescence and confocal microscopy of bovine and human articular cartilage to examine the expression of connexin 43 hemichannels, which are known to act as mechanosensitive ATP release channels in other cell types. In

addition, we examined the expression of purine receptors and of primary cilia, which are putative mechanosensory organelles.

Bovine articular chondrocytes were found to express both primary cilia and connexin 43 hemichannels. Hemichannel expression was confirmed by positive labelling of the extracellular domain. Furthermore, at least 50% of the primary cilia were decorated with connexin 43. Studies using human articular cartilage revealed that only chondrocytes within the superficial zone expressed connexin 43. Chondrocytes throughout all zones of the tissue expressed purine receptors P2X2, P2X4, P2X7 and P2Y1, whereas P2Y2 was expressed only by superficial zone cells.

These studies suggest that the chondrocyte primary cilium may serve as a strain amplifier in that its distortion activates ATP release via connexin 43 hemichannels. The extracellular ATP may then bind to P2 receptors, initiating a purinergic mechanotransduction pathway leading to alterations in extracellular matrix synthesis.

(64) Chondrocytes Isolated from Different Cartilage Tissues Show Different Gene Expression Patterns

Vonk L.A.¹, Zandieh Doulabi B.², Huang C.L.², Kroeze R.J.², Helder M.N.², Bank R.A.²

¹Academic Center for Dentistry Amsterdam (ACTA), Dept Oral Cell Biology, Amsterdam, The Netherlands

²VU University Medical Center, Dept Orthopaedic Surgery, Amsterdam, The Netherlands

The main problem in tissue engineering of cartilage is an appropriate production of extracellular matrix (ECM). Remarkably, relatively little is known about the relative gene expression levels of genes involved in ECM homeostasis between chondrocytes in different tissues, and whether gene expression is stable in alginate beads. Alginate is often used as a vehicle to culture chondrocytes.

Chondrocytes were isolated from nucleus pulposus, annulus fibrosis, meniscus, and articular cartilage from goats. Before seeding (day 0) and after 18 days in alginate beads, cells were subjected to real-time PCR to determine the gene expression of collagen type I (COL1A2), heat shock protein 47 (HSP47), aggrecan (AGC1) and collagenase 3 (MMP13). Data were normalized against the three housekeeping genes with the formula $3\sqrt{(\text{HPRT} \times \text{UBC} \times \text{YWHAZ})}$.

Major differences exist in mRNA levels at day 0 with respect to COL1A2 and AGC1 between the different chondrocyte populations. Culturing of chondrocytes in alginate beads for 18 days resulted in an increase of COL1A2 and HSP47, a major increase of MMP13, and a decrease in AGC1. All chondrocyte phenotypes reacted in a similar way on their stay in alginate beads.

We demonstrated (1) that chondrocytes isolated from different tissues show different gene expression levels and (2) that gene expression levels are dramatically changed in alginate beads. It is likely that a cartilage ECM is being formed that differs from its natural counterpart. Furthermore, the induction of MMP13 is worrying, as this enzyme is involved in collagen degradation in osteoarthritis.

(65) Chondrogenic Induction of Canine Mesenchymal Stem Cells in High Density Co-culture with Chondrocytes: Potential Applications in Regenerative Medicine

Csaki C.¹, Mobasheri A.², Matis U.³, Putz R.¹, Shakibaei M.¹

¹Musculoskeletal Research Group, Institute for Anatomie, LMU Muenchen, Pettenkoferstr. 11, D-80336 München, Germany

²Division of Veterinary Medicine, University of Nottingham, Sutton Bonington Campus, LE12 5RD, United Kingdom

³Clinic of Veterinary Surgery, LMU Muenchen, Veterinärstr. 13, D-80539 München, Germany

To overcome the clinical problem of unsatisfactory articular cartilage repair with *in vitro* expanded chondrocytes (de-differentiated cells), we investigated cartilage formation in a high density co-culture of primary isolated, differentiated chondrocytes with pluripotent progenitor cells. The hypothesis being that the primary isolated un-differentiated chondrocytes induce chondrogenesis in the progenitor cells.

Canine bone marrow mesenchymal stem cells (MSCs) were cultured in a ratio of 1:1 in monolayer and high density systems with primary isolated chondrocytes. High density cultures of pure MSCs and pure chondrocytes were used as controls. The high density cultures were evaluated with light-, electron microscopy, and immunofluorescence.

After seven days in high density cultures with pure chondrocytes we observed large and round cells surrounded by a well developed extracellular matrix (ECM). After the same period of time in cultures of pure MSCs (without pure chondrocytes) cell lysis was observed. In contrast to this, in co-cultures the cells appeared organised. This organisation showed similarities to the structure of cartilage and perichondrium *in vivo*: flat, elongated fibroblast-like cells on the rim of the cartilage-nodules and large, round cells surrounded by ECM in the middle of the nodules. Furthermore, we observed expression of collagen type II, cartilage specific proteoglycans and integrins in co-cultures as we did in cultures of pure chondrocytes.

We assume that in this co-culture model the chondrocytes provide an inductive differentiation signal to the MSCs activating the appropriate chondrogenic signaling pathways and therefore making externally added growth and differentiation factors redundant.

(66) Clinical Testing of Tissue Engineered Bone Substitute for Regeneration of Damaged Periodontal Bone Tissue

Maličev E.¹, Kregar-Velikonja N.¹, Marolt D.², Vihar K.³, Kreft M.E.⁴, Rode M.³

¹Educell d.o.o., Letališka 33, Ljubljana, Slovenia

²Blood Transfusion Centre of Slovenia, Šlajmerjeva 6, Ljubljana, Slovenia

³Health Care Centre of Ljubljana, Ljubljana, Slovenia

⁴Medical Faculty of Ljubljana, Institute for Cell Biology, Vrazov trg 2, Ljubljana, Slovenia

Tissue engineering technology enables replacement and regeneration of lost bone tissue with autologous bone substitutes. We performed clinical trial to test effectiveness of the use of cellularised bone substitutes as an alternative procedure for bone augmentation in periodontology.

Grafts were prepared by autologous osteoblasts, isolated from alveolar bone proliferated *in vitro* and seeded on a hydroxyapatite carrier material. Grafts for control group were prepared in same way, only without cells. Osteogenic character of cells was examined by real time RT-PCR analysis of specific genes alkaline

phosphatase, osteopontin and osteocalcin and mineralization of extracellular matrix. Implantation of grafts was performed using periodontal guided tissue regeneration technique.

Clinically, regeneration of tissue was evaluated by examination of clinical status, measuring of periodontal indexes (SBI, GI, etc.) and X-ray analysis. Three months after surgery preliminary results of X-ray analysis indicate bone formation in test group, which is not seen in control group. Clinically there were no significant differences between both test groups in this time point.

Application of autologous tissue-engineered grafts exhibited no signs of adverse effects of the treatment and can be efficiently used in periodontal applications to promote alveolar bone regeneration.

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(67) Clinical Trial of Bone Marrow Stromal Stem Cell Implantation for Refractory Fracture Non-Union: Results from the First Patients

Bajada S., Harrison P., Kuiper J.H., Balbouzis T., Ashton B.A., Cassar Pulicino V., Ashammakhi N., Richardson J.B.

Institute of Science and Technology in Medicine (ISTM), Keele University, Keele, Staffordshire ST5 5BG, United Kingdom
Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, Shropshire, SY10 7AG, United Kingdom

Introduction: This research aims to study the efficacy of Bone Marrow Stromal Stem Cell (BMSSC) implantation on healing of refractory fracture non-union.

Methods: Twelve patients (9M and 3F), age range 38 to 76 years (mean 49.9), with non-union resistant to multiple previous treatments (mean 3.75 procedures) were included in a study approved by the local Research Ethics Committee. Four had tibial and eight had femoral fractures. The patients were admitted for harvesting of stromal cells by bone marrow aspiration from the iliac bone. BMSSC were culture-expanded for three weeks to an average of 5×10^6 cells. At implantation the non-union site was decorticated and BMSSC added to synthetic bone graft substitutes (different types) on one side of the fracture (medial or lateral) according to randomisation. The other side received bone graft substitute alone. The side treated with cells was blinded to patient, surgeons and radiologists. Standard radiographs were taken and evaluated by experienced musculoskeletal radiologists. The extent of callus formation on each side was recorded. In equivocal cases computerized tomography (CT) was also obtained.

Results: No patient developed systemic complications related to the procedure. On follow-up callus formation was present in 9 of 12 patients. Callus was more marked on the cell implantation side in 6/12 patients (50%), control side in 1/12 (8.3%) and equal in 2 patients. 3 patients showed no change.

Discussion: These findings suggest that implantation of BMSSC can enhance bone formation in persistent nonunion. A larger randomized controlled trial will follow to test this new treatment.

(68) Close Dependence of Fibroblast Growth on Collagen Scaffold Matrix Stiffness

Hadjipanayi E., Mudera V., Brown R.A.

University College London (UCL), Tissue Repair and Engineering Centre, Institute of Orthopaedics, Stanmore Campus, London, HA7 4LP, United Kingdom

Human dermal fibroblasts in free floating collagen matrices divide very little, though this may increase when the matrix is under tension. This study investigated the idea that this is in fact a cell response to substrate stiffness (rather than tension) with important implications for engineering of tissues. Adult HDFs were seeded into free floating, attached gels and plastic-compressed (PC) gels made with a range of collagen densities (giving a stiffness series). Mechanical properties were measured using a Dynamic mechanical analyser (Perkin Elmer); cell number by Alamar Blue assay. In maximum density PC gels (> 99% fluid loss) cell proliferation was rapid. Expansion was seeding density-dependent and with no lag period (contrast with 4 day lag for nonPC gels). Doubling time was reduced from 6 to 2 days. Inhibition of cell growth was seen at 1 million cells/ml. HDF growth was related to matrix stiffness such that gels at a range of compression levels (0 to 75% fluid removal) supported increasing proliferation rates from 5 to 3 days doubling times and this in turn related to increasing matrix elastic modulus. HDF quiescence in uncompressed gels was reversible, such that compression of gels at 1 and 5 days initiated HDF growth.

We conclude that collagen matrix stiffness regulates proliferation of fibroblasts (a duro-response) with important implications for the design and regulation of engineered connective tissues such as dermis based on collagen and hydrogel-based scaffolds.

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(69) Co-culture Bioreactor Design for Skeletal Tissue Engineering

Michael V., Gittings J., Turner I., Chaudhuri J., Ellis M., Waters S., Cummings L., Goodstone N., Cartmell S.

Institute of Science and Technology in Medicine, University of Keele, Stoke-on-Trent, Staffordshire ST4 7QB, United Kingdom
Sports, Medical and Materials Group, Department of Mechanical Engineering, University of Bath, Bath BA2 7AY, United Kingdom
Department of Chemical Engineering, University of Bath, Bath BA2 7AY 4, United Kingdom
Division of Applied Mathematics, School of Mathematical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom

Bioreactor design for the culture of tissue-engineered constructs is essential for the production of functional tissue. Most of these bioreactors are optimized for a single cell type e.g. osteoblasts or chondrocytes. Co-cultures of cartilaginous and bone tissue together have been shown to have beneficial effects compared to monocell cultures. We have developed a bioreactor enabling culturing of bone and cartilage cells together. This system allows nutrient administration via two separate methods to allow scale up of larger tissues. It incorporates an online monitoring system to allow for

quality control of the developing tissue construct. The bioreactor also has the potential of mechanical load application. It consists of two separate bone and cartilage culture chambers that are then assembled together with the bone/cartilage separated by a silicone rubber membrane. This enables different culture environments to be provided to these related but different tissue types. Porous calcium-phosphate (15 mm diameter×10 mm height) and Chitosan hydrogel (15 mm diameter×6 mm height) are used to support the bone/cartilage cells respectively. A peristaltic pump delivers the perfusing media whilst a PLGA porous fibre running through the bone scaffold delivers extra nutrients via diffusion. Through this fibre, a temperature/oxygen sensor is introduced to the samples, allowing monitoring of cellular activity. Non-invasive lactate and glucose culture media analysis also allows further quantification. Mathematical modelling of this bioreactor has been performed elucidating the fluid flow parameters throughout the construct in the bioreactor. This sophisticated co-culture bioreactor has the potential to significantly improve the functionality of tissue-engineered osteochondral plugs.

(70) Co-culture of Mesenchymal Stem Cells with PC-12 Cells Protects both from Apoptotic Cell Death

Wang H., Liu T.Q.

Dalian R&D Center for Stem Cell and Tissue Engineering, Dalian University of Technology, China

Mesenchymal stem cell (MSC) administration is thought to promote neuronal survival in neurodegenerative brain disorders. The aim of this study was to determine whether the observed survival effect was due to diffusible factors secreted by murine MSCs and whether diffusible agents produced by PC-12 cells (rat pheochromocytoma cells) could sustain mMSCs *in vitro*. PC-12 cells were treated by serum-free medium to induce apoptosis, and then co-cultured with mMSCs in serum-free medium for 48 hours using transwell culture dishes so that the two cell types were exposed to the same culture media condition without direct cell contact.

Cell viability was measured by WST-8 (CKK-8) assay and cell apoptosis was quantified by fluorescence-activated cell sorter (FACS) analysis after stained with annexin V and propidium iodide (PI). After 48 hours of serum deprivation, the cell viabilities of PC-12 cells and mMSCs in serum-free medium were reduced to 50% and 60 %, respectively. In contrast, the viability of PC-12 cells and mMSCs increased by 20% under co-culture condition. Meanwhile, the early apoptosis (annexin V+ and PI-) of PC-12 cells and mMSCs after 48 hours of serum deprivation increased to 6 and 5 times, respectively, whereas co-culture could reduce early apoptosis of PC-12 cells and mMSCs by 30% and 25%. The results of this study suggest that co-culture of mesenchymal stem cells with PC-12 cells protects each other from apoptotic cell death *in vitro*. The protective effects might be mediated through diffusible factors secreted from mMSCs and PC-12 cells.

(71) Collagen Fiber Based Anterior Ligament Grafts

Weber M.¹, Seefried L.¹, Kirsch L.², Stehle J.¹, Heymer A.¹, Hurschler C.², Krohne G.³, Nöth U.¹

¹Orthopaedic Center for Musculoskeletal Research, Division of Tissue Engineering, University of Wuerzburg, Germany

²Orthopaedic Department, Hannover Medical School, Germany

³Division of Electron Microscopy, Biocenter of the University of Wuerzburg, Germany

Introduction: Tissue engineering of anterior cruciate ligament (ACL) grafts provides an auspicious alternative to overcome the problems associated with current strategies for ACL reconstruction, such as donor site morbidity and limited availability. This study aims to develop a fully biological compound ACL-constructs *in vitro*.

Methods: Collagen fibers were manually isolated from rat tails and sterilized by γ -irradiation. Compound ACL-constructs were engineered using human bone marrow-derived stem cells (hMSCs), a collagen type I hydrogel (Arthro Kinetics), porcine small intestine submucosa (SIS), and collagen fibers. Tensile strength of single collagen fibers and complete ACL-constructs was scrutinized on a material testing machine. The microstructure of the ACL constructs was visualized by histochemistry and scanning electron microscopy (SEM).

Results: Following γ -irradiation, maximum tensile forces of single collagen fibers increased to 8.3 ± 3.5 N as compared to 4.3 ± 0.9 N in native fibres. Failure of compound ACL-constructs occurred at 24.5 ± 0.8 N. SEM of single collagen fibers revealed a predominantly parallel orientation of the fibrils with slightly twisted fibril bundles. Comparing untreated and γ -irradiated collagen fibers, no structural differences were observed. Furthermore, compound ACL-constructs displayed good bonding of collagen hydrogel, collagen fibers and SIS. Histochemistry indicated elongated fibroblast-like cells oriented parallel to the wavy collagen fibers.

Discussion: Gamma-irradiation appears to increase the maximum tensile force of collagen fibers from rat tail without affecting their microstructure as detected by SEM. The combination of SIS with hMSCs, collagen hydrogel, and collagen fibers seems to be a promising approach for *in vitro* fabrication of ACL-constructs.

(72) Collagen Type II and Nucleus Pulposus Cells: Synergistic Effects on Chondrogenic Differentiation of Adipose-Derived Stem Cells

Lu Z.F.¹, Wuisman P.I.¹, Zandieh Doulabi B.¹, Bank R.A.², Helder M.N.¹

¹Department of Orthopaedic Surgery, VU University Medical Center (VUMC), Amsterdam, The Netherlands

²Business Unit Biomedical Research, TNO Quality of Life, Leiden, The Netherlands

Background: Degenerative intervertebral disc disease (DDD) is one of the most prevalent causes for chronic low back pain. Injection of adipose stem cells (ASCs) into the nucleus pulposus (NP) might be used for regeneration of the affected disc.

Aim: To investigate whether collagen type II and NP cells, main constituents of the nucleus, affect chondrogenic differentiation of ASCs in a 3D environment.

Methods: Human ASCs were cultured in type I or II collagen gels, and the effects of this 3D environment on chondrogenesis-related gene expression (collagen types I, IIA, IIB and X, Aggrecan) were

determined after culturing for 4–21 days. Subsequently, these data sets were compared to results from similar ASC cultures, but now co-cultured with NP cells in 0.4 μm -transwell systems. ASC mono-layer cultures served as controls.

Results: Without NP cells, collagen type I and II gels had similar effects on ASC differentiation; only (early) upregulation of collagen type X, but not of aggrecan and collagen types I, IIA and IIB were observed. However, in ASC-NP co-cultures, collagen type X was up-regulated in both collagen type I and II gels, whereas collagen type I gels only induced early collagen type IIA gene expression in ASCs. Strikingly, collagen type II gels could act synergistically with NP cells on chondrogenic (collagen type IIA and IIB) gene expression in ASCs.

Conclusion: This study demonstrates that collagen type II and NP cells act synergistically on ASC differentiation toward the NP phenotype, suggesting ASC injection to be a feasible approach for disc regeneration treatment modalities.

(73) Collagen Type V Enhances Matrix Contraction by Fibroblasts Seeded in 3D Collagen Constructs

*Bains M.¹, Lambert S.², Mudera V.¹

¹Tissue Repair and Engineering Centre, IOMS, University College London, United Kingdom

²Royal National Orthopaedic Hospital, Stanmore, United Kingdom

Introduction: Extracellular matrix is composed of many components that play a crucial role in modulating cytomolecular cellular activity. Connective tissue contains several types of collagen with concentrations varying in different tissues. We investigated the effect on cytomolecular cellular contraction of ECM using composite 3D collagen constructs seeded with human shoulder fibroblasts.

Methods: Primary explants of fibroblasts from human shoulder capsule and ligament were obtained using standard tissue culture techniques. Contraction of fibroblasts seeded in 3-D collagen constructs (i) 100 % Collagen Type I and (ii) Composite constructs (90% Type I & 10% Type V) were quantified over 24 hours in free floating constructs and using a culture force monitor (CFM).

Results: Human shoulder capsule and shoulder ligament fibroblasts did not contract free floating 3D constructs or register measurable contraction on the CFM over 24 hours with evidence of attachment and viability over 24 hours. Free floating composite constructs of Collagen Type I and V (ratio 9:1) showed a significant decrease in area compared to collagen type I constructs. Significant greater force generation was measured in the composite constructs compared to constructs containing only collagen I over 24 hours.

Conclusion: The enhanced contraction of fibroblasts in composite constructs may be mediated through integrins (1) or a change in collagen fibril diameter (2) due to the addition of Collagen Type V. Electron microscopy studies are underway to quantify changes in 3D topography of collagen fibril diameter. We have shown that composite constructs (collagen I and collagen V) may play an im-

portant role in modulating cytomolecular cellular contractile responses mimicking changes that occur during wound healing and tissue regeneration.

References

1. Berendsen et al. 2006 Matrix Biology 25(8): 515–522.
2. Spiess et al. 2007 Microsc Res Tech 70(1): 18–25.

Acknowledgements: Royal National Orthopaedic Hospital, Stanmore for funding the research.

(74) Collagen-Chitosan Polymeric Scaffolds for the *In Vitro* Culture of Human Adipose Tissue-Derived Stem Cells

Zhu Y.X., Song K.D., Wen P.F., Liu T.Q.

Dalian R & D Center for Stem Cell and Tissue Engineering, Dalian University of Technology, No. 2 Linggong Road, Ganjingzi District Dalian, China

Introduction: A cell-based tissue engineering technique has been proved to be one of the most promising alternative therapies. Adipose tissue-derived stem cells (ADSC) have been proved to be a potential clinically useful source of cells for cellular therapy and tissue engineering. Thus developing a three-dimensional scaffold suitable to the growth of ADSC is necessary in tissue engineering.

Methods: Chitosan was fully mixed with type I collagen with different volume ratio 9:1, 7:3, 5:5, 3:7 and 1:9 (collagen:chitosan), then freeze-dried and cross-linked. The microstructure of scaffolds was observed via scanning electron microscopy (SEM). Pore size, bibulous ability, water content and interval porosity were measured before and after cross-linking. The enzyme degradation was performed to evaluate the degradability of the scaffold *in vitro*. And the biocompatibility of ADSCs with scaffolds was observed by SEM and hematoxylin and eosin stain.

Results: The pore size of scaffolds decreased with the decrease of collagen concentration. The cross-linking treatment could enhance the pore size and decrease the bibulous ability of the scaffold, while the interval porosity has no significant difference. *In vitro* enzyme degradation indicated that uncross-linked composite scaffolds had faster degradation rate than cross-linked. The affinity of scaffold with ratio 7:3 to ADSCs was better than that of other scaffolds.

Discussion and conclusion: Comprehensively considering pore size, bibulous ability, interval porosity, biodegradation and biocompatibility of all kinds of scaffolds, we can conclude that the affinity of composite scaffolds with volume ratio 7:3 to ADSCs was better than that of other scaffolds, and suitable to the growth of ADSCs in three dimensions.

(75) Combining Osteogenic and Endothelial Progenitors in a Hybrid Bone Construct

Fedorovich N.E., Haverslag R.T., Verbout A.J., Alblas J., Dhert W.J.A.

Department of Orthopaedics, University Medical Center Utrecht, Utrecht, The Netherlands

Low survival of tissue engineered bone grafts due to insufficient blood supply limits development of larger grafts. To stimulate vascularisation, we intend to coimplant endothelial and osteogenic progenitors. Endothelial progenitor cells (EPCs), a potent source of endothelial cells, form tubular structures *in vitro* and can connect to the vascular system of the host after implantation [1]. The aim of this study was to isolate and characterize goat EPCs and to study their interaction with osteogenic cells *in vitro*.

Mononuclear cells were isolated from either goat bone marrow or peripheral blood and cultured in EGM2 medium. The cells were characterized by immunocytochemistry after 4 days for the uptake of acetylated LDL and binding of isolectin B4, and after 14 days for PECAM-1 expression. Vascular networks were evaluated with the angiogenesis assay. In co-culture experiments, EPCs were labeled with CFSE and co-embedded with osteoprogenitors in Matrigel for up to 3 days.

90% percent of goat EPCs stain double positive for early endothelial markers. gEPCs exhibit high proliferation rates, and express PECAM-1 after 14 days of culture. gEPC form networks when seeded on a Matrigel surface, although these are less elaborate than those formed by HUVEC and GVEC. Networks formed in co-cultures are derived from gEPC as evidenced by CFSE fluorescence. Low ratios of gEPC:osteoprogenitors were most effective for network formation.

We can efficiently isolate gEPCs that express early endothelial markers and become positive for late endothelial marker PECAM-1 within two weeks. *In vitro* gEPC form networks on and in Matrigel, and are responsible for network formation in co-cultures with osteoprogenitors. gEPC are potentially useful to stimulate vascularisation *in vivo* in bone tissue engineering.

Reference

1. Ribatti D. (2007); Leuk Res 231(4):439–44.

(76) Combining Time-Lapsed μ CT Monitoring and Mechanical Stimulation in Bone Tissue Engineering

Hagenmüller H.^{1,2}, Merkle H.P.², Meinel L.², Müller R.¹

¹ETH Zurich, Institute for Biomechanics, Wolfgang-Pauli-Str. 10, 8093 Zurich, Switzerland

²ETH Zurich, Institute of Pharmaceutical Sciences, Wolfgang-Pauli-Str. 10, 8093 Zurich, Switzerland

We present a novel system for tissue engineering of bone *in vitro*, combining time-lapsed micro-computed tomography (μ CT) monitoring and mechanical stimulation. Custom-made culture chambers are kept inside an incubator and can be taken out of the incubator for time-lapsed *in situ* imaging inside a μ CT desktop system under sterile conditions. Like this, bone morphological parameters of individual constructs can be monitored throughout the culture period. Furthermore, the culture chambers can be mounted into a custom-made mechanical stimulation unit which can apply direct mechanical compression to the constructs. The mechanical stimulation unit allows compressive loads with a force resolution of 0.1 N, displacement steps as small as 100 nm over a travel range of 6 mm, and frequencies up to 10 Hz. When applying compression to the constructs, the stiffness of the individual samples gets re-

corded. Each culture chamber hosts one loaded sample and one non-loaded, paired control sample, which is exposed to the same environment except for the compression. To prove the functionality of the system, human mesenchymal stem cells were cultured on silk fibroin scaffolds in osteogenic medium for 42 days in the system. The cells fully maintained viability and showed continuous depositing of bone-like tissue. μ CT imaging revealed trabecular-like morphologies and stiffness measurements indicated an increase in the constructs' stiffness throughout culture. Different loading regimes resulted in different bone deposition rates. The presented system can be used to adapt the culture environment (i.e. mechanical stimulation, duration of culture or medium composition) to the current state of tissue deposition—individually for each sample. Like this, image-guided feedback control in tissue engineering of highly functional bone may become possible.

(77) Comparative Cell Engineering Using Differential in Gel Electrophoresis

Kantawong F., Burchmore R., Dalby M.J.

Centre for Cell Engineering, Division of Infection & Immunity, IBLS, Glasgow University, Glasgow, G12 8QQ, United Kingdom

Bone tissue engineering is considered to hold potential for the production of bone fragments for use in repair of bone defects. Here, human osteoprogenitor cells isolated from femoral heads have been used to represent the osteogenic cell types found within the bone marrow. In tissue engineering, the use of bioresorbable scaffolds is attractive as over time the scaffold will be resorbed and bone tissue will form and replace the degraded material. In this study, microgrooves embossed in polycaprolactone (bioresorbable) have been used as a bioactive scaffold material and the cells/scaffolds have been cultured in a flow bioreactor. The osteoblast progenitor cells were seeded onto microgrooved and flat PCL sheets and then cultured at 37°C in static environment for 1 week before moving into the flow chambers. The flow system was maintained for 3 weeks at 37°C and protein extraction was performed before analysis with differential in gel electrophoresis (DIGE). Proteins of interest were picked from the resulting gel and analyzed by Mass Spectroscopy (MS). Identification of proteins that are modulated when cells are grown on grooved materials will highlight pathways that are involved in the cellular response to a patterned culture surface. Down-regulation of cytoskeletal protein, vimentin, actin and tubulin has been noted which suggests that the cells are responding to mechanotransductive stimuli from the topographies. This information will contribute to understanding the factors that are important in scaffold design and culture conditions.

(78) Comparative Micro-CT Analysis of Two Different Scaffolds Seeded with Mouse or Human Mesenchymal Stem Cells Undergone Myoblastic Differentiation

Giuliani A.³, Komlev V.³, Moroncini F.¹, Pennesi G.², *Rustichelli F.³, Tasso R.², Torrente Y.⁴

¹Department of Materials and Environment Engineering and Physics, Polytechnic University of Marche, Ancona, Italy

²Department of Oncology, Biology, and Genetics; University of Genova, Genova, Italy

³Department of Sciences Applied to Complex Systems; Marche, Polytechnic University of Marche, Ancona, Italy

⁴Fondazione IRCCS Ospedale Policlinico di Milano, Italy

Although tissue engineering approaches for skeletal muscle regeneration have been proposed [1], new efforts to better understand the reciprocal interactions between cells and bioscaffolds should be made. In this context, we compared how human mesenchymal stem cells (hMSC) or murine MSC (mMSC), induced toward myoblastic differentiation, interacted with two different materials used as scaffolds, namely PLGA/PLLA (50/50) (Concordia Fibers, USA) and Tissucol (Baxter SpA, Italy) [2, 3].

In particular 50×103 MSC, undergone myoblastic differentiation using 5-Azacytidine (10 µM/l) and labelled with Endorem[®], were seeded in each scaffold. After 15 days in culture, the cells/scaffold combinations were processed for cell growth and differentiation analysis by Thymidine uptake test and molecular analysis by RT-PCR, respectively. Twin cells/scaffold combinations were formalin-fixed for micro-CT analysis using synchrotron radiation (ESRF Beamline 05, Grenoble, France) [4]. Cell growing rate was higher for cells seeded on Tissucol, but the micro-CT images were clearer for cells seeded on the PLGA/PLLA scaffold, depicting a three-dimensional organization of cells around the fibres.

Although not conclusive, these results support the possibility of using stem/precursor cells and bioscaffold for the repair of damaged skeletal muscle.

References

1. A.D. Bach et al. "Skeletal muscle tissue engineering". J Cell Mol Med, 8(4), 2004, 413–422.
2. A.S. Saxena et al. "Skeletal muscle tissue engineering using isolated myoblasts on synthetic biodegradable polymers: preliminary studies". Tissue Eng, 5(6), 1999, 525–531.
3. G. Bilic et al. "Human preterm amnion cells cultured in 3-D collagen I and fibrina matrices for tissue engineering purposes". Am J Obstet Gynecol, 193(5), 2005, 1724–1732.
4. Y. Torrente et al. "High-resolution X-ray microtomography for three-dimensional visualization of human stem cell muscle homing". Febs Letter, 580, 5759–5764, (2006).

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(79) Comparison of Autologous Full Thickness Gingival and Skin Substitutes for Wound Healing

Gibbs S., Waaijman T., Vriens B.P., Van den Hoogenband H.M., De Boer E.M., Scheper R.J.

VU University Medical Centre, Department of Dermatology, De Boelelaan 1117, Amsterdam, The Netherlands

Background: Tissue-engineered products should maintain the characteristics of the original tissue. For example: skin is an example of orthokeratinized epithelium and oral gingival is an example of parakeratinized epithelium. The aim of this study was to develop an autologous full thickness gingival and skin substitutes suitable for clinical applications.

Methods: Skin and gingival substitutes were constructed under identical culture conditions from 3 mm punch biopsies isolated

from the upper leg or gingival tissue respectively. Both consisted of reconstructed epithelia on acellular dermis re-populated with fibroblasts. To compare the characteristics of the original and reconstructed tissue, morphological observations and expression of differentiation markers (keratins-6, 10, 17 and stratum corneum precursors: involucrin, loricrin and SKALP) were determined. Skin and gingival substitutes were transplanted onto therapy resistant leg ulcers or tooth extraction sites in order to determine their effects on wound healing.

Results: The tissue engineered constructs maintained many of the characteristics of the original tissues from which they were derived. The skin substitute was orthokeratinized, and the gingival substitute was parakeratinized. Transplantation of skin ($n=19$) and gingival substitutes ($n=3$) resulted in accelerated wound healing.

Discussion: This study emphasizes the necessary to closely match donor sites with the area to be transplanted. Our results represent a large step forward in the area of clinical applications in oral tissue engineering which have until now greatly lagged behind skin tissue engineering.

(80) Comparison of Mammalian (Murine, Human) Stem Cell Derived Engineered Lung Tissue with Native Lung

Nichols J.E., Niles J.A., Bynam S., Cantu A., McClure M., Fung C., Cortiella J.

University of Texas Medical Branch, Department of Internal Medicine, Division of Infectious Diseases, Department of Anesthesiology Laboratory of Tissue Engineering and Organ Regeneration and Department of Microbiology and Immunology, Galveston, Texas, United States of America

The theory behind regenerative therapy is to obtain the ideal cell or set of cells that will integrate itself within the existing lung structure. One method of identifying the ideal cell is to create an *ex vivo* system that is capable of recreating the native organ on a specialized or smart matrix. Within the lung a specific cell type that is capable of regenerating the lung has not been identified. Recently we have identified a heterogeneous population of lung progenitor cells that are capable of generating lung tissue when grown on a three dimensional polymer.

Our research shows that the mammalian (murine, human) engineered bronchiole-alveolar tissue is self-sustaining in culture within a rotary bioreactor for at least 8 weeks. Histologic sectioning reveals a three-dimensional architecture consistent with that of cells found at the bronchiole-alveolar junction. The engineered tissue contains cell types and in numbers that correspond with the distribution of *in vivo* lung. Cell types examined included but were not restricted to Type I and Type II pneumocytes, Clara cells, endothelial cells, neuroendocrine cells, smooth muscle and mucin secreting cells or goblet cells.

The engineered tissue emulates the role of the lung parenchyma in the modulation of the lung proteome which is the dynamic collection of specialized lung proteins. Expression of protein products was evaluated by immunocytochemistry, immunoprecipitation and western blotting and included inflammatory mediators such as cytokines, leukotrienes and endothelins, as well as chemotactic

mediators such as chemokines, defensins, surfactant proteins A, and D and cathelicidin LL37.

(81) Compressed Collagen Gels as Cell Delivery for Bladder Tissue Regeneration

Engelhardt E., Stegberg E., Foroughi F., Mudera V., Brown R.A., Adam M., Frey P., Wurm F.

Laboratory of Cellular Biotechnology, EPFL, Lausanne, Switzerland

Cystoplasty using gastrointestinal segments is the common treatment for patients with end-stage bladder disease. To overcome complications associated to this treatment, successful cell-based approaches have been reported. We explored a new scaffold consisting of a plastic compressed collagen gel. This approach allows engineering of biomimetic cell-independent constructs with excellent mechanical properties. Such scaffold has been used to grow smooth muscle (SMC) and urothelial (UC) cells, the final goal being the repair of human bladder tissue. We showed that SMC can be integrated successfully inside the compressed collagen gels without significant changes in viability and that both cell types individually and as co-culture proliferate and stay alive up to day 14 (end of the culture). These results support the use of compressed collagen gels as cell delivery for bladder tissue regeneration.

(82) Computational Simulation of Cell Migration Under Different Substrate Stiffness

Moreo P., García-Aznar J.M., Doblaré M.

University of Zaragoza, Spain

It is well established that tissue cell behavior is influenced by the stiffness of the substrate. For instance, cell migration speed is higher on softer substrates and cells exert higher tension on stiffer substrates and migrate preferentially towards stiffer regions [1]. However, most of existing computational models do not consider this influence. In this work, we propose a simple mathematical model that reproduces many of the effects of substrate stiffness on cell locomotion and cell-traction forces.

The main features of the model are two: (a) cells “detect” substrate rigidity exerting traction forces consuming a fixed amount of energy; (b) besides the typical flux terms considered in most of cell migration models (diffusion, chemotaxis, haptotaxis), an additional term containing the divergence of the cell-exerted stress tensor is included. This term appears in a natural way when the flux term is derived following rigorous thermodynamic arguments [2].

2D finite element simulations of cell migration on planar substrates have been performed using the proposed model and the results indicate that the model is able to reproduce with sufficient accuracy, in agreement with experimental observations [1], the influence of substrate rigidity on the magnitude of cell forces and cell speed migration as well as the preference of adherent cells towards stiffer regions (“mechanotaxis” or “durotaxis”).

References

1. Discher D.E. et al. (2005). Tissue cells feel and respond to the stiffness of their substrate. *Science* 310(5751):1139–43.

2. Doblaré M. and García-Aznar J.M. (2005). On the numerical modelling of growth, differentiation and damage in structural living tissues. *Arch. Comput. Meth. Engng.* 11(4):1–45.

(83) Construction of PIRE2-AcGFP1-CD Eukaryotic Expression Plasmid and Its Expression in Bone Marrow Mesenchymal Stem Cells

Chen Y.Q.¹, Song F.¹, Ma X.H.¹, Ge D.¹, Liu T.Q.¹, Ma Y.F.², Cui Z.F.³

¹Dalian R & D Center for Stem Cell and Tissue Engineering, Dalian University of Technology, Dalian, 116023, China

²Biochemical Division, Basic Medical School, Dalian University of Medicine, Dalian, 116021, China

³Department of Engineering Science, Oxford University, Parks Road, Oxford OX1 3PJ, United Kingdom

Objective: To construct a eukaryotic expression plasmid pIRE2-AcGFP1-CD and to transfect it into bone marrow mesenchymal stem cells.

Method: The cytosine deaminase gene was obtained from *E. coli* JM109 genome DNA by Polymerase Chain Reaction (PCR). The fragment was cloned into pMD19-T vector. Restriction enzyme BamHI/XhoI digestion analysis and DNA sequence analysis showed that CD gene was identical with the published sequence. Constructing the pIRE2-AcGFP1-CD plasmid and identified by restriction enzyme BamHI/XhoI digestion analysis. Transfecting the bone marrow mesenchymal stem cells through liposome-mediated method. The expression of CD was confirmed by fluoroscopy after transfection.

Results: We cloned CD gene and eukaryotic expression plasmid was constructed. Bone marrow mesenchymal stem cells were successfully transfected by CD gene.

Conclusion: The transfected bone marrow mesenchymal stem cells displaying green fluorescence were observed under fluorescence microscope. It is possible that bone marrow mesenchymal stem cells to become the ideal vector in gene therapy.

(84) Controlled Angiogenesis by Rapid FACS-Purification of Myoblasts Expressing Defined VEGF Levels

Misteli H., Fueglistaler P., Wolff T., Gianni-Barrera R., Gurke L., Heberer M., Banfi A.

Basel University Hospital, Department of Surgery and Department of Research, Basel, Switzerland

Vascular Endothelial Growth Factor (VEGF) gene delivery is a powerful strategy to induce angiogenesis in the treatment of ischemic diseases or for the vascularization of tissue-engineered grafts. However, its microenvironmental dose must be controlled, as VEGF remains bound around each producing cell (Ozawa, JCI 2004). To achieve homogeneous controlled expression *in vivo*, we developed a Fluorescence Activated Cell Sorting (FACS)-based method to allow the rapid purification of genetically-engineered myoblasts expressing predictable levels of VEGF.

Myoblasts were transduced with a retrovirus expressing VEGF164 linked to a truncated CD8a marker in a bicistronic cassette. Differences in VEGF expression were reflected by the cell-surface amount of CD8a, quantified on individual live cells by FACS.

The transduced population expressed very heterogeneous transgene levels, but VEGF and CD8 expression were linearly correlated ($R^2 = 0.897$) in individual clones (range = 2–142 ng VEGF/ 10^6 cells/day). A clone expressing the highest safe VEGF level (40 ng, inducing only normal angiogenesis *in vivo*) was selected as reference. Cells expressing similar CD8a levels (and therefore of VEGF) were purified from the primary population using 2 different gate sizes. *In vivo*, the heterogeneous population always caused angioma growth. In contrast, the sorted populations caused robust normal angiogenesis, with double vessel length-density compared to controls and normal capillary diameter distribution. Long-term experiments showed that the angiogenic response was stable after 4 months, without adverse effects.

Therefore, FACS-purification of homogeneous populations expressing predictable VEGF levels completely prevented angioma growth, allowing safe and efficient angiogenesis *in vivo*. In principle, this technology can allow controlled expression of any therapeutic gene of interest in different kinds of progenitors.

(85) Cultivation of Human Meniscus Cells on Porous Silk Scaffolds

Kessler O., Falco C., Thomsen A., Thoma S.

Stryker Orthopaedics, Thalwil, Switzerland CellGenix Technologie, Freiburg, Germany

Introduction: Meniscal lesions fail to heal spontaneously. Therefore, cell-based reconstructions of the menisci with scaffolds made of silk should provide initial support for cell attachment, matrix deposition and cell differentiation. In this study, cultivation of human meniscus cells on silk scaffolds under GMP conditions was evaluated.

Material and Methods: Biopsies were harvested from the meniscus of 8 patients. Cells were cultivated under standard conditions (37°C, 5% CO₂) with the use of serum free media. After 3rd passage density increased between 1.2×10^7 to 4.4×10^8 cells. The expanded meniscus cells were then seeded (1×10^6 meniscus cells/cm²) onto the surface of porous silk scaffolds with pore sizes (800 µm) and cultured for 6, 10 and 14 days. All procedures were done under GMP conditions. Cells were directly stained with fluorescein or phycoerythrin conjugated monoclonal antibodies specific for CD44, CD105, CD31, CD34, CD95, CD29, CDE25 and AS02 or indirectly stained with unconjugated monoclonal antibodies specific for aggrecan and collagen type II. FACS was used to evaluate the antigenic phenotype of meniscus cells.

Results: The antigen-expression of meniscus cells in monolayer-culture was negative for CD25, CD29, CD31, CD34 and positive for CD44, CD95 und AS02. Expression of aggrecan and collagen-type II was only positive in 3-dim alginate-bead-culture. Immunofluorescence microscopy of scaffolds seeded with meniscal cells revealed a high rate of emission of green fluorescence and therefore a high rate of vital meniscal cells within the scaffolds. HE staining showed a colonialization of multilayer cells within the pores.

Discussion: As shown with histology the meniscal cells formed colonies within the deeper pores and expressed extracellular matrix which demonstrates beginning remodeling.

(86) Culture and Expansion of Mesenchymal Stem Cells in Air-Lift Loop Hollow-Fiber Membrane Bioreactor

Li X.Q., Liu T.Q., Song K.D., Zhu L., Ge D., Guan S., Ma X.H., Cui Z.F.

Stem Cell and Tissue Engineering Lab of Dalian University of Technology, Dalian, 116024, Liaoning, China

Mesenchymal stem cells (MSCs), having the multi-differentiation potential, are the promising “seed cells” in tissue engineering and ideal target cells in gene therapy. However, MSCs are in limited quantity, which makes it necessary for them to be expanded *in vitro*.

Here, MSCs of passage 4 (P4) were mixed with 3 mg·mL⁻¹ type I collagen with the density of 5×10^5 cells·mL⁻¹ and inoculated into hollow-fiber membranes (HFM), where the gel with MSCs formed after an hour in an incubator at 37°C, 5% CO₂. Then the gel with MSCs inside HFM was inoculated into air-lift loop bioreactor (ALB) to culture MSCs in three dimensions. Finally, cell number, growth curves and the metabolite were detected; the expanded cells were identified through antibodies CD29, CD44 and CD45 by flow cytometer and through inducing them into osteoblasts, chondrocytes and adipocytes.

The results showed that in the air-lift loop hollow-fiber membrane bioreactor (ALHFMB), O₂ concentration kept constant; MSCs metabolized prosperously and expanded about 20-fold within 7 days; the most of the expanded cells were CD29 and CD44 positive and CD45 negative; after being induced, the expanded cells still reserved the strong multi-differentiation potential.

This study first explored the feasibility of three-dimensional dynamic expanding MSCs in ALHFMB, and the expanded cells still keep the capacity of extensive self-renewal and multilineage differentiations. Therefore, it is a safe protocol for MSCs to be cultured inside HFM with type I collagen for expansion, because shear stress can be avoided when they are in dynamic culture in bioreactors.

(87) Customized Fibrin Matrices and Tissue Engineering

Weijers E.M., Joosten L., Kwok-Cheung E., Van Hinsbergh V.W.M., Bank R.A., De Maat M.P.M., Koolwijk P.

VU Medical Center, Department of Physiology, Amsterdam, The Netherlands

One major challenge of tissue engineering is to accelerate the process of neovascularization of the scaffold *in vivo*. Furthermore control of cell growth and differentiation on suitable matrices is important in generating tissue grafts. Fibrin, important in hemostasis, also provides a biodegradable extracellular matrix for cell invasion and angiogenesis, and is biocompatible for several cell types via integrin (e.g. RGD sequences) and non-integrin binding sites.

Fibrinogen is synthesized as a high molecular weight form (HMW) containing two A α , two B β and two γ -chains. Partial degradation of one C-terminus of the alpha chains in the circulation

results in a low molecular weight form (LMW) and degradation of both alpha chains results in LMW fibrinogen, which lacks RGD-sequences and sites for lateral aggregation, plasminogen and factor XIII binding. The aim of this study is to develop customized matrices containing variants of fibrin(ogen), which can influence the growth of cells and the angiogenic response.

The fibrinogen variants with different molecular weight were either isolated from plasma-derived fibrinogen or recombinantly produced. Using expression in CHO-cells, the HMW fibrinogen form and three major variants of LMW fibrinogen were produced. In addition fibrin(ogen) variants with a decreased fibrinolysis rate were recombinantly generated. Fibrin matrices were prepared with single fibrinogen variants or mixtures thereof and the interactions with endothelial cells were studied, e.g. adhesion, proliferation, migration and tube formation, providing information about *in vitro* culturing of cells on fibrin matrices and the angiogenic properties of these modified matrices.

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(88) Cyclic Tensile Strain Influences the Chondrogenic Differentiation of Mesenchymal Stem Cells Via Stretch-Activated Ion Channels

McMahon L.A.¹, Campbell V.A.^{1,2}, Prendergast P.J.¹

¹Trinity Centre for Bioengineering, School of Engineering, Trinity College, Dublin, Ireland

²Department of Physiology, School of Medicine, Trinity College, Dublin, Ireland

The interaction of mechanical loading and choice of biomaterial are important for MSC differentiation in the engineering of a suitable construct for cartilage defects. The hypothesis of this project is that cyclic mechanical tensile loading will regulate the chondrogenic differentiation of MSC-seeded collagen-GAG scaffolds in the presence of TGF- β 1. Additionally, it is proposed that the mechanotransduction process is regulated via stretch-activated (SA) ion channels.

MSCs were harvested from Wistar rats, characterised and seeded into collagen-GAG scaffolds [1]. Culture conditions included (i) free-swelling controls, (ii) uniaxial clamped or (iii) uniaxial clamped + cyclic tensile strain (10% at 1 Hz for 7 days) in the presence of 10 ng/ml TGF- β , following 7 days of static culture. SA-ion channels were blocked using 10 μ M gadolinium. [35S] sulphate incorporation was used as a measure of the rate of GAG synthesis, and cell-mediated contraction of free-swelling constructs (\pm TGF- β 1) was quantified over 14 days.

TGF- β 1 treatment significantly increased GAG synthesis compared to untreated controls ($p < 0.01$, $n = 9$), which corresponded to an increase in cell-mediated contraction of the scaffolds ($26.9 \pm 2.2\%$ vs. $15.4 \pm 1.15\%$, $p < 0.01$, $n = 4$). Uniaxial clamping significantly reduces GAG synthesis compared to free-swelling controls, indicating the importance of cell-mediated contraction for differentiation ($p < 0.01$, $n = 10$). Cyclic tensile loading significantly increased GAG synthesis compared to clamped controls ($p < 0.01$, $n = 10$) demonstrating the mechanoresponsiveness of the cells. Blocking SA-ion channels significantly reduced GAG synthesis compared to the loaded group ($p < 0.05$, $n = 5$) demonstrating that SA-ion channels are involved in transducing me-

chanical signals during dynamic loading to control the chondrogenic differentiation of MSCs in this 3D environment.

Reference

1. O'Brien FJ et al., (2005), *Biomaterials*, 26(4):433–441.

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(89) Human Dermal Fibroblasts with Mutations in COL3A1 Genes and Their Effect on Mechanical Force Generation within 3D Collagen Matrices

Guirav N.¹, Mudera V.², Bradey M.², Pope F.M.¹

¹Northwick Park Institute of Medical Research (NPIMR), Northwick Park Hospital & St Marks Trust (Y Block), Watford Road, Harrow, HA1 3UJ, United Kingdom

²UCL, Tissue Repair and Engineering Centre, Institute of Orthopaedics and Musculoskeletal Sciences, London, HA7 4LD, United Kingdom

Adequate secretion of extra cellular matrix constituents, within a 3 dimensional scaffold is crucial in the development of a fully functional tissue engineered construct for *in vivo* implantation. However, the role played by various ECM components is still poorly understood, especially in environments subject to mechanical strain. We used the CFM model (Eastwood M et al, 1994) and fibroblasts derived from patients with Ehlers Danlos syndrome Type IV (COL3A1) mutation to study their cyto-mechanical behaviour in 3D collagen scaffolds compared to normal fibroblasts.

COL3A1 mutations lead to the production of faulty Type III collagen. Zoppi et al (2004) have suggested that COL3A1 mutations alter the organisation of collagens and fibronectin in the ECM, which we hypothesize, will have a direct effect on the cyto-mechanical behaviour of these cells.

Results show differences in the measurable force outputs of EDS type IV cell lines compared to normal fibroblasts. The average peak force generated was 106 dynes for normal and 284 dynes for EDS IV cells. The rate of force generation particularly at 3–4 hours was also higher for EDS IV cells compared to normal fibroblasts. Cell mediated contraction occurs during the first 4 hours as the cells attach to the matrix.

Differences in expression of cytoskeletal proteins, Actin and Vimentin were detected, suggesting that subtle organisational differences induced by unstable Type III collagen play a key role in cell attachment and force generation. These findings are critical as Type III collagen is the first collagen detected *in vivo* during wound healing reported to be mechanically weak.

(90) Degradation and Mechanical Property Assessment of Scaffolds for Vascularised Tissue Regeneration

Clark M.E., Felstead P.J.C., Everitt N.M., Scotchford C.A., Grant D.M.

University of Nottingham, School of M3, Bioengineering Group, University Park, Nottingham, NG7 2RD, United Kingdom

Vascularisation of engineered scaffolds for the replacement of cartilage and bone is one of the main obstacles facing the field of regenerative medicine. The absence of an adequate blood supply results in hypoxic conditions and ultimately necrosis of the engineered tissue. It is necessary to develop a method of connecting tissue engineered products to the existing system through biodegradable three dimensional scaffolds. Knowledge of the degradation profile of the scaffold is related to its isotropic/anisotropic structure is required.

Work presented here is part of a study examining the degradation of isotropic and anisotropic scaffolds. The change in mechanical properties over the degradation period was assessed with and without gamma irradiation.

PLGA tubes not subjected to gamma irradiation did not undergo hydrolysis over the 10 week degradation period most likely due to having an initial molecular weight of 300 kg mol⁻¹, since water ingress into the structure did occur. Gamma irradiation significantly reduced the molecular weight of the PLGA tubes to 150 kg mol⁻¹, although no significant change in storage modulus (59 MPa) was seen when oscillated at 1 Hz using DMA. A significant loss in molecular weight (160 to 140 kg mol⁻¹) and stiffness (54 to 30 MPa) of the gamma irradiated samples was seen after 1 week of hydrolysis, with a more gradual loss seen thereafter.

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(91) Dehydrothermal Crosslinking of Collagen-GAG Scaffolds

Haugh M.G., Jaasma M.J., O'Brien F.J.

Department of Anatomy, Royal College Surgeons in Ireland, 123 st. Stephens Green, Dublin, Ireland

Tissue-engineered bone provides an alternative source of bone grafts. Collagen-GAG (CG) scaffolds with a porosity of 99% have shown great potential as a construct on which to culture bone cells [1]. However, the mechanical properties of CG scaffolds are currently not suitable for use as a bone graft substitute. Therefore, the goal of this study was to investigate the effects of dehydrothermal (DHT) crosslinking on the mechanical properties of the scaffolds.

Scaffolds were produced via a freeze-drying process [2]. DHT crosslinking was then carried out at a pressure of 0.05 bar. DHT duration and temperature were varied between 24 and 96 hours and 105°C and 180°C, respectively. Compressive and tensile testing were carried out on hydrated samples to determine elastic modulus. Fourier transform infra-red (FT-IR) microscopy was used to analyse denaturation of the collagen and the level of crosslinking within the scaffolds [3, 4].

Results show that the modulus can be substantially increased using DHT. A 2-fold increase in compressive modulus and a 3-fold in tensile modulus were achieved by increasing the temperature and duration of crosslinking. Denaturation was found to increase with increasing temperature and exposure period. Ongoing research is investigating the effects of crosslinking on degradation and the activity of cells seeded onto the scaffolds.

References

1. Farrell et al. (2006). *Tissue Eng.* 12. pp. 459–468.
2. O'Brien et al. (2005). *Biomaterials.* 25. pp. 1077–1086.

3. Gordon et al. (1974). *Macromolecules.* 7. pp. 954–957.
4. Wang et al. (2003). *Biomaterials.* 24. pp. 3213–3220.

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(92) Delivery of Therapeutic Gene ENOS via a Fibrin Scaffold Enhances Wound Healing in a Compromised Wound Model

Breen A., Dockery P., O'Brien T., Pandit A.

National Centre for Biomedical Engineering Science, Department of Anatomy and Regenerative Medicine Institute, National University of Ireland, Galway, Ireland

Deficient nitric oxide (NO) production is a cause of impaired wound healing [1]. NO is synthesized in cells by endothelial nitric oxide synthase (eNOS) enzyme. The study aims to deliver an adenovirus gene vector encoding eNOS (AdeNOS) via a fibrin scaffold to a compromised wound model resulting in sustained, local production of NO from host cells and enhanced wound healing, compared with adenovirus alone.

Hyperglycaemia was induced in eight New Zealand White rabbits by alloxan administration. After 7 days, four 6 mm punch biopsy wounds were created on each ear and the following treatments administered randomly: fibrin containing AdeNOS, fibrin alone, AdeNOS alone and no treatment. Animals were sacrificed at 7 and 14 days, and Griess assay carried out to assess nitrite production, in addition to stereological analysis in order to quantify rate of epithelialisation of the wounds and surface density of blood vessels, from histology sections.

Griess assay showed that the fibrin-AdeNOS group was the only treatment to show more nitrite production than normal unwounded tissue. Fibrin-AdeNOS treatment showed a greater rate of re-epithelialisation than any other group. At 14 days, the surface density of blood vessels is less in the fibrin-AdeNOS group than all other groups, indicating maturation of healing. It is concluded that fibrin containing AdeNOS enhances gene transfer to tissue compared with direct delivery of AdeNOS alone, achieving an enhanced wound healing response.

Reference

1. Schaffer MR, et al. *Am. Coll. Surg.* 1997; 184: 37–43.

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(93) Design and Fabrication of Bionic Scaffolds with Polygradient Microstructure

Liulan L., Huicun Z., Qingxi H., Li Z., Fang M.

Rapid Manufacturing Center, Shanghai University, Shanghai 200444, China

The repairing of bone defects has been an intractable problem in clinic therapy. The development and application of tissue engineering in recent years has provided a new solution for this problem. A novel method of repairing bone defects based on Mimics and tissue engineering was studied in this paper. At first the STL model of defective bone was obtained through Mimics' image processing and 3D reconstruction technology. Then the restoration's

CAD model of bone defect was constructed by 3D modeling software UG. The shape of the restoration was just the shape of the bionic bone scaffold to prepare. Based on the microstructure of real bone and tissue engineering's requirement to bionic scaffold, an indirect method combining stereolithography and slurry casting was used to prepare bio-ceramic scaffolds with polygradient microstructure. With the analysis of the physical, mechanical and biological characteristics of the bionic scaffolds, it was found that these scaffolds perform enough strength and was suitable for cell adherence and multiplying. These results show that the method of repairing bone defects based on Mimics and tissue engineering was feasible in clinic therapy.

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(94) Design of a Bioreactive Composite Scaffold for Improved Vascular Connexion of Tissue-Engineered Products—VASCUPLUG

Seifert B., Missirlis Y., Knedlitschek G., Grant D., Cao Y., Alonso M.J., Keri G.

¹GKSS Forschungszentrum, Institute of Polymer Research, Kantstr. 55, 14513 Teltow, Germany

²University Patras, Biomedical Engineering Lab, Rion 26500, Patras, Greece

³Forschungszentrum Karlsruhe, P.O. Box 3640, 76021 Karlsruhe, Germany

⁴University of Nottingham, School of MMME—Bioengineering Group, University Park, Nottingham, NG7 2RD, United Kingdom

⁵Karolinska Institutet—MTC, Nobelsvägen 16, Box 280, 171 77 Stockholm, Sweden

⁶University of Santiago de Compostela, School of Pharmacy, Campus Sur, 15782 Santiago de Compostela, Spain

⁷Cooperative Research Center, Semmelweis University Budapest, Rippl-Ronai u. 37, P.O. Box 131, Budapest 5, Hungary

Tissue engineering is a main challenge in regenerative medicine. But engineered tissues today in many cases lack appropriate connexion to the vascular system and the surrounding tissue at the implantation site. Hence, they suffer from malnutrition and low gas exchange leading to necrosis. To overcome this bottleneck the strategic objective of VASCUPLUG project is to develop a novel three-dimensional scaffold structure for improved vascularisation of tissue-engineered products and connexion to host tissue.

The key issues of this project are selection and processing of suitable polymers to design and process a special 3D scaffold that meets the demands of formation of new blood vessels, whilst accommodating the sterilisation and degradation processes of the polymers. Extensive testing of the components and the 3D system with respect to biocompatibility and functionality *in vitro* is a necessary issue of the work. Incorporation of bioactive factors to promote the formation of blood vessels and proof of their action are further topics as well as the final proof of concept implemented in an animal model.

The expected impact of the VASCUPLUG project will be a composite scaffold giving rise to evolving vessels that allow

proper vascular connexion to the surrounding tissue in the course of wound healing. The complex bunch of requirements for a functional vascularisation of tissue-engineered products calls for a strong interdisciplinary co-operation of chemists, biologists, engineers, physicists and physicians and depends upon pronounced cross-frontier collaboration as reflected in the VASCUPLUG consortium.

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(95) Design of an Automated Culture System for Serial Passages of Human Myoblast Cells

Kino-oka M., Muneyuki Y., Chowdhury S.R., Taya M.

Osaka University, Div Chemical Engineering, Osaka 5608531, Japan

Osaka University, Dept Biotechnology, Osaka 5650871, Japan

For clinical application using cells and tissues, cell processing, including steps such as isolation of target cells, serial subcultures for cell expansion (passages) and tissue culture for structural differentiation, is required. These complicated steps were conducted by experienced operators in cell pressing center (CPC) in hospitals and companies. For the labor saving with retaining high safety management in CPC, automation and operational simplification are critical issues. The culture system (bioreactor system) is considered to realize not only these requirements but also moderate culture environment, leading to stable cell processing. In the present study, we developed the automated system of passage in serial monolayer cultures of human myoblast cells.

To realize the higher performance of subcultures, the influences of seeding density and confluence degree on growth were investigated quantitatively in terms of cell attachment, division and proliferative cell population. Culture at lower seeding density ($< 1.0 \times 10^3$ cells/cm²) induced the spatial heterogeneous distribution of cells in the flask, causing higher frequency of myotube formation which were lower ability of attachment on the surface. The quantitative analyses of these cellular behaviors determined the seeding density and attainable confluence degree during one passage as the initial and boundary conditions, respectively. Bioreactor system which could manage two serial subcultures by monitoring of confluent degree was constructed. The automated operation with intelligently determining the time for passage was successfully performed without any loss of growth activity, compared with manual operation using conventional flasks. These results suggested that the stable cell process was realized in the system.

(96) Design Simplifications in Corneal Engineering

Elsheikh A.

Division of Civil Engineering, University of Dundee, Dundee, United Kingdom

The cornea has a complex microstructure which may be difficult to replicate using tissue engineering approaches. The construction of the stroma with its 200–250, multi-directional layer structure poses particular difficulties [1, 2]. Numerical simulation based on the finite element method was used to estimate the effect of

introducing simplifications in the structure of the stroma, in particular the reduction of stromal layers to 10 and the restriction of collagen fibril orientation within layers to the superior-inferior (vertical) and temporal-nasal (horizontal) directions. The numerical models were built to simulate constructions approximating natural and simplified structures. They were subjected to (i) a uniform posterior pressure simulating intraocular pressure, and (ii) a concentric anterior load representing external loads creating corneal bending, such as those encountered in tonometry. The loads were increased gradually until a corneal apical deformation of more than 2 mm had been achieved. The numerical analysis indicated the superiority of the natural stromal structure, especially under anterior loads creating corneal bending. Under these loads, corneal models with the simplified structure deformed significantly more (by > 40%) than the natural structure. However, under intraocular pressure—the normal pressure loading on the cornea—the difference between the mechanical behaviour of the two models was insignificant; below 1%.

References

1. Müller L.J., et al. A new three-dimensional model of the organization of proteoglycans and collagen fibrils in the human corneal stroma. *Exp. Eye Res.* 2004;78:493–501.
2. Daxer A, Fratzl P. Collagen fibril orientation in the human corneal stroma and its implication in keratoconus. *IOVS*, 1997; 38(1):121–1293.

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(97) Designing Extracellular Matrix Scaffolds by Dynamic Culture of Fibroblasts

Aulin C.¹, Foroughi F.², Hilborn J.¹

¹Uppsala University, Polymer Chemistry, Lagerhyddsvägen 1, 75237 Uppsala, Sweden

²UCL, Institute of Orthopaedics and Musculoskeletal Science, Brockley Hill, Stanmore, Middlesex, HA7 4LP, United Kingdom

Our bodies are constantly exposed to different sorts of mechanical forces, from muscle tension to wound healing. Connective tissue adapts its extracellular matrix (ECM) to changes in mechanical load and the influence of mechanical stimulation on fibroblasts has been studied for a long time [1, 2]. When exposed to forces, fibroblasts are known to respond with expression and remodeling of ECM proteins, in particular collagen type I [3]. In this study the effect of dynamic culture conditions on human dermal fibroblasts was evaluated in terms of deposition and remodeling of ECM, with the aim of producing an ECM based scaffold. The fibroblasts were grown on compliant polymer supports either in a bioreactor with a pulsating flow or under static conditions. By applying dynamic culture conditions, the collagen deposition on the polymer supports increased fivefold. Scanning electron microscopy showed that polymer fibers were well integrated with cells and ECM and alignment along the polymer fibers was observed. Scaffold design should aim at creating structures that can help guiding the cells to form new, functional tissue. The presented system may present a new way of producing designed extracellular matrix based scaffolds for tissue engineering.

References

1. Grinnell, F., (1994) Fibroblasts, myofibroblasts and wound contraction, *J. Cell. Biol.* 124:401–404.
2. Brown, R.A., Prajapati, R., McGrouther D.A., Yannas, I.V. and Eastwood, M., (1998) Tensional homeostasis on dermal fibroblasts: Mechanical responses to mechanical loading in three-dimensional substrates, *J. Cell. Phys.* 175:323–332.
3. Lambert, C.A., Soudant, E.P., Nusgens, B.V., Lapière, C.M., (1992) Pretranslational regulation of extracellular matrix macromolecules and collagenase expression in fibroblasts by mechanical forces, *Lab. Invest.* 66:441–451.

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(98) Determination of Surrogate Connective Tissue Biomarkers in Erosive Osteoarthritis

Braun M., Hulejová H., Olejárová M., Pavelková A., Šenolt L., Pavelka K.

Institute of Rheumatology, Na Slupi 4, Prague, 128 50, Czech Republic

Introduction: Erosive osteoarthritis (EOA) affects predominantly interphalangeal joints of the hands. Contrary to non-erosive OA, it results in joint destruction and increased local inflammation. Determination of bone, cartilage, synovial, and/or inflammatory parameters may help to differentiate between erosive and non-erosive forms.

Patients and methods: Urine and serum samples from 47 patients with hand EOA (mean age 64 years) and 24 with non-erosive OA (mean age 62 years) were included into this cross-sectional study. Collagen cross-link such as urinary deoxypyridinoline (DPD) was determined by IMMULITE, pentosidine (PEN) in serum and urine by HPLC. Serum levels of hyaluronic acid (HA) were measured by ELISA and C-reactive protein (CRP) by immunoturbidimetry.

Results and discussion: Significant increase of both DPD ($p = 0.01$) and HA ($p = 0.01$) concentrations was demonstrated in patients with EOA contrary to patients with non-erosive disease. Only insignificant elevation of PEN in urine and serum was detected in patients with EOA. However, no difference in serum CRP was found between both OA groups. In conclusion, markers of bone turnover and synovial inflammation may represent surrogate markers to x-ray to differentiate between erosive and non-erosive osteoarthritis. Longitudinal studies are indispensable to demonstrate association of the markers with OA progression and therapeutic effects.

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(99) Developing an Engineered Urinary Stroma *In Vitro*

Baker S., Southgate J.

Jack Birch Unit for Molecular Carcinogenesis, Department of Biology, University of York, Heslington, YO10 5YW, United Kingdom

Stromal cells generate the contractile force required for bladder voiding *in vivo*, however, when established *in vitro*, smooth muscle contractile protein expression was reduced and proliferation increased. This study aimed to investigate the effects of two and three-dimensional culture on the phenotype of human urinary tract stromal cells, based upon the hypothesis that increased cell:cell over cell:substrate interaction would promote differentiation.

A panel of smooth muscle differentiation markers was developed from the *in situ* phenotype and used to characterise cells *in vitro*. In monolayer cultures serum deprivation and the addition of TGF β 1 inhibited proliferation and stimulated contractile marker expression.

Sub-micron scale electrospun polystyrene scaffolds were used to study stromal cell biology in three-dimensions. Stromal cell cultures on electrospun scaffolds exhibited a rate of proliferation comparable to monolayer cultures. However, whilst serum deprivation reduced proliferation in electrospun scaffolds, it did not stimulate stromal differentiation. The lack of differentiation was potentially due to carboxyl groups on the scaffold surface, which when combined with the higher surface area of scaffolds, led to the retention of inhibitory serum factors.

When intact biomimetic urothelial sheets were seeded on stromal cell seeded scaffolds the urothelium showed polarisation and stratification and the neo-bladder tissue formed a basement membrane of collagen type IV. The combination of scaffold technology and basic cell biology permitted the study of heterotypic cell:cell interactions in three-dimensional cultures and has led to the creation of a novel tool for the observation of tissue interactions.

(100) Developing Nanoscale Parameters for Nanofibrous Scaffolds

Ortega S., Henry J.A., Pandit A.

Department of Mechanical and Biomedical Engineering, National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland

Electrospinning can produce nanofibrous scaffolds. Scaffold degradation and cellular infiltration have been shown to be dependent on pore size [1], whilst fibre alignment is crucial in certain tissue engineering applications, such as scaffolds for skeletal muscle [2]. This study proposes a framework for the architectural characterisation of electrospun scaffold parameters which will be relevant to understand the influence of the nanoscale in the development of clinical applications. Images of electrospun scaffolds were acquired using scanning electron microscopy (SEM) and exported to image analysis software (Image Pro[®] Plus, Media Cybernetics, UK) to obtain Fast Fourier Transform (FFT) algorithms. A method for transforming FFT visual outputs into numerical data was developed and automated through software development. Specifically, software was developed for the automation of the following parameters: fibre orientation, spatial distribution of fibre orientation, pore size, pore aspect ratio and spatial distribution of pore size. Software validation involved comparing two pictures with known fibre alignment, drawn with computer software (AutoCAD[®]). Upon validation, the software quantified the aforementioned parameters of an electrospun porous network. The obtained results indicate that the algorithm can quantify the scaffold parameters which are relevant for applications of electrospun networks up to five fibres thick. Future research involves enhancing

the software to calculate these parameters for unlimited fibre thickness.

References

1. Henry J.A., et al. J Biomed Mater Res 2007(A).
2. Riboldi S.A., et al. Biomaterials 2005; 26: 4606–4615.

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(101) Development of a 3-Dimensional *In Vitro* Model to Study Reactive Gliosis Following Nervous System Injury

East E., Golding J.P., Phillips J.B.

The Open University, Department of Biological Sciences, Walton Hall, Milton Keynes, MK7 6AA, United Kingdom

Injury to the spinal cord results in the formation of a glial scar which is associated with inhibition of axonal regeneration. One of the major limitations of research into improving repair strategies is the lack of a cell culture model that accurately recapitulates the complex *in vivo* situation. Our aim is to develop an effective model to address this need.

Astrocytes in the undamaged CNS express low levels of GFAP, but following injury exhibit a reactive phenotype exemplified by GFAP up-regulation. Primary glial cell cultures were analysed in 2D monolayers and 3D collagen gels for GFAP expression. In 2D cultures 73.4 \pm 4.0% of cells were GFAP positive, whereas 40.7 \pm 3.5% were immunoreactive for GFAP in 3D collagen gels. As 3D astrocyte cultures more closely modelled the *in vivo* situation we used this model to investigate the response of astrocytes to dorsal root ganglia cells (DRGs). Dissociated DRGs were labelled with CellTracker[™], seeded onto astrocyte-populated collagen gels and maintained in culture for 5 days. Astrocytes near the DRG interface showed marked GFAP up-regulation and adopted a reactive morphology which was observed up to 1 mm away.

Astrocytes in 3D culture exhibit a lower basal level of reactivity than cells grown in monolayer, providing a system in which stimulation of activation can be investigated. This model provides a useful tool for investigating triggers of reactive gliosis, as demonstrated by the response observed to cells found at the inhibitory interfaces formed following damage to the spinal cord.

(102) Development of a Calcium-Phosphate Coated Collagen Scaffold

Al-Munajjed A.^{1,2}, Gleeson J.^{1,2}, O'Brien F.^{1,2}

¹Department of Anatomy, Royal College of Surgeons in Ireland, Dublin, Ireland

²Trinity Centre of Bioengineering, Trinity College, Dublin, Ireland

Previous investigations have shown that collagen, a normal constituent of bone and cartilage, shows excellent biological performance as a scaffold, especially in cell growth and adhesion (1). However it has insufficient mechanical properties for implantation in a load-bearing defect. The objective of this study was to develop a collagen/calcium-phosphate composite scaffold for use in bone tissue engineering.

Pure collagen scaffolds were produced with a final freeze-drying temperature of -40°C as previously described (2). The scaffolds were soaked for two different exposure times (1 and 22 h) in $\text{NaNH}_4\text{HPO}_4$ and CaCl_2 solutions of three different concentrations (0.1 M, 0.5 M and 1.0 M) as previously described (3), producing six different composite scaffold types. Pure collagen scaffolds served as control. The porosity (using μCT) and the compressive moduli (unconfined hydrated compression) were measured to describe the scaffolds.

All scaffolds showed a significantly increased stiffness relative to control. The groups treated with the 0.5 M concentrations showed the highest values (70 times increased stiffness). The porosity of all coated scaffolds was reduced relative to the pure collagen scaffold. After the 0.1 M solutions porosities of 80% and after the 0.5 M solutions porosities of 70% could be seen in the scaffolds' centre. Further investigations concerning the structural composition, the permeability and the biological compatibility are ongoing.

References

1. O'Brien, F.J. et al., (2005) *Biomaterials*, 26 433–41.
2. O'Brien, F.J. et al., (2004) *Biomaterials*, 25 1077–86.
3. Al-Munajjed, A.A. et al., (accepted) *Studies in Health Technology and Informatics*.

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(103) Development of a Human Hemicornea for Pharmacotoxicological Testing

Builles N.¹, Bechetoille N.², Justin V.¹, André V.², Pellegrini G.³, Auxenfans C.¹, Hulmes D.J.S.⁴, Damour O.¹

¹Cell and Tissue Bank, Hospices Civils de Lyon, Lyon, France

²Engelhard-Coletica, Lyon, France

³Veneto Eye Bank Foundation, Ospedale Civile di Venezia, Venice, Italy

⁴Institut de Biologie et Chimie des Protéines, CNRS/Université Claude Bernard Lyon 1 UMR5086, IFR 128 Biosciences Lyon-Gerland, Lyon, France

We have developed a hemi-cornea suitable for pharmacotoxicological testing. It comprises a stroma of collagen/GAG/chitosan in the form of a porous matrix. Seeded with keratocytes at passage 5 this substrate was cultured two weeks in a medium selected to optimise proliferation and phenotype fidelity so as to obtain a 3-dimensional stromal equivalent (SE). These SE were then seeded with cornea epithelial cells and cultured for 1 week in immersed conditions, then for a further two weeks at the air/liquid interface giving rise to an hemicornea. Histological images reveal the migration and proliferation of keratocytes throughout the stroma thickness with a well developed pluri-stratified epithelium on the surface. Immunohistological studies showed: the basement membrane stained for laminin 5; the matrix deposited by keratocytes stained for collagen type I in the neostroma and collagen type VI around the keratocytes. Transmission electron microscopy showed an ultrastructural organization of the ECM comprising periodically striated collagen fibrils organised in bundles, with a mean fibril diameter and an inter-fibril spacing very close to the values in human stroma. These parameters are considered of prime importance for transparency. Lastly we observed pseudopod-like structures

and intercellular junctions typical of keratocytes. The three dimensional hemicorneas contained in supporting inserts are now being used for pharmacotoxicological testing.

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(104) Development of a Heterotypical Corneal Substitute Using Oral Mucosa Keratinocytes

Alaminos M., Sanchez-Quevedo C., Garzon I., Gonzalez-Andrades M., Muñoz-Avila J.I., Moreu G., Lobo M., Campos A.

Departments of Histology (Group of Tissue Engineering) and Stomatology, University of Granada, Spain. University Hospital San Cecilio, Granada, Spain

Introduction: Tissue engineered human corneas could eventually be used to replace damaged corneas without the present disadvantages or corneal transplantation (1). In this work, we describe the use of oral mucosa keratinocytes to replace corneal epithelium in bioengineered corneas.

Materials and Methods: To develop a heterotypical substitute of the human cornea, we generated primary cell cultures of corneal stromal keratocytes and oral mucosa keratinocytes from small tissue biopsies. First, we generated a stroma substitute made of cultured keratocytes entrapped in a gel of human fibrin and 0.1% agarose. Then, cultured oral mucosa epithelial cells were grown on top. Stratification of the epithelial cell layer was promoted by using an air-liquid culture technique. Corneal substitutes were analyzed by light and electron microscopy. To identify genes up or downregulated in heterotypical corneal equivalents, total RNA was extracted and hybridized to Affymetrix U133 plus 2.0 microarray chips.

Results and Discussion: Structural analysis confirmed the presence of a stratified epithelium on top of the corneal constructs, with numerous desmosomes and microvilli. These constructs expressed higher levels of genes related to epidermic development (KRTHB1, SCEL), cell proliferation control (RARRES1, DLEU1), cell metabolism (TF, TCN1, UGT2B15) and immune system (HLA-DQB1, SERPINB4, TNFSF11). Our results suggest that heterotypical corneal constructs show several similarities with normal native corneas and could eventually be used to replace these organs.

Reference

1. Alaminos et al. (2006). Construction of a complete rabbit cornea substitute using a fibrin-agarose scaffold. *Invest Ophthalmol Vis Sci*. 47:3311–3317.

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(105) Development of an Immunoassay for Determination of Adsorbed Fibronectin Levels on Bio-functionalised Substrates

Heywood H.K., Lee D.A.

Queen Mary University of London, Medical Engineering division of Engineering, Mile End Road, London, E1 4NS, United Kingdom

By promoting cell adhesion and associated changes in cytoskeletal organisation, surface bound adhesion proteins such as fibronectin (fn) also modulate spreading, migration, proliferation and viability. The controlled deposition of fn has consequently been explored as a means for directing such fundamental cellular processes *in vitro* and may prove essential to the success of biomimetic materials employed in tissue engineering and regenerative medicine. However, the direct quantification of surface bound adhesion molecules by accessible immunological methods has been limited, particularly by non-linearity of the assay response.

We have developed an immunoassay for surface bound fn, using a monoclonal anti-human fn mouse IgG targeted to the fn cell binding domain, fn III (Sigma Aldrich, Poole, UK) and linked to a fluorogenic enzyme substrate detection stage that can be read within a multiwell plate using a fluorimeter. The influence of fn surface density ($0.08\text{--}10\text{ mg}\cdot\text{cm}^{-2}$) on the assay output parameter was investigated on 1 cm^2 silicon specimens and used to define the dynamic range of the immunoassay. Test specimens were compared to reference materials prepared within this range, to determine the amount of adsorbed fn.

An excellent linear fit between fn density and assay response was obtained for densities up to $0.25\text{ mg}\cdot\text{cm}^{-2}$. Although significantly less than the maximal density for a fibronectin monolayer, literature indicates that this functional range of assay performance is most relevant to the field of surface biomimetics. Further modifications with potential to enhance the linear range will be discussed.

(106) Development of an Inflatable Surgical Device for Endoscopic Treatment Using Oral Mucosal Epithelial Cell Sheets

Ohki T.^{1,2}, Yamato M.², Maeda M.², Murakami M.^{2,3}, Okano T.², Yamamoto T.¹

¹Department of Surgery, Institute of Gastroenterology, Tokyo Women's Medical University, Japan

²Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Japan

³Graduate School of Science and Engineering, Waseda University Tokyo, Japan

Introduction: Large oesophageal endoscopic mucosal resection (EMR) often requires subsequent balloon dilation to prevent post-operative oesophageal constriction. We have previously developed a novel method to transplant autologous oral mucosal epithelial cell sheets to promote oesophageal wound healing after EMR (Gut, 1704-1720, 2006). Although we have shown the successful endoscopic transplantation of two cell sheets, it remains difficult to transplant cell sheets to circumferential ulcerative areas. We therefore developed a novel device for the endoscopic delivery and transplantation of cell sheets.

Methods: Oral mucosal epithelial cells harvested from beagle dogs were seeded on temperature-responsive dishes and cultured for 2 weeks. Oral mucosal epithelial cell sheets were harvested by simple temperature-reduction. Four individual cell sheets were then simultaneously transplanted to a circumferential oesophageal ulceration of a deceased pig using a novel inflatable device.

Results: An inflatable balloon was attached to the exterior of standard oesophageal EMR-tube having an outer cylindrical cover. For

transplantation, the device was carefully moved to the ulcer site by endoscopy. Ten minutes after inflation of balloon we macroscopically confirmed complete circumferential transplantation of the cell sheets. Iodine staining indicated that all of the cell sheets were successfully attached to the oesophageal ulcer. Histological results also confirmed that the epithelial cell sheets were attached to the remnant submucosal layers.

Conclusions: Our results show that this novel balloon device completely enables the transplantation of several tissue-engineered cell sheets by endoscopy.

(107) Development of Bespoke Bone Scaffolds Using Mesenchymal Stem Cells and Bioceramics

Dyson J.¹, Genever P.², Dalgarno K.³, Wood D.¹

¹Division of Oral Biology, Leeds Dental Institute, University of Leeds, Leeds, LS2 9LU, United Kingdom

²Biomedical Tissue Research, Department of Biology, University of York, York, YO10 5YW, United Kingdom

³School of Mechanical and Systems Engineering, University of Newcastle, Newcastle, NE1 7RU, United Kingdom

Selective laser sintering (SLS) is a rapid prototyping technology which is used to produce intricate 3D scaffolds to match the exact geometry of a patient's graft. Apatite-wollastonite (A-W) is a bioactive glass-ceramic which shows excellent biocompatibility *in vivo*, has similar mechanical properties to bone and can be processed by SLS. We have used human mesenchymal stem cells (MSCs) to populate porous A-W scaffolds produced by SLS to create bespoke biocompatible bone replacement structures. Confocal and scanning electron microscopy (SEM) were used to demonstrate that MSCs adhered to, retained viability and penetrated the pores of A-W scaffolds over 21 days of static culture. We identified a significant increase ($p < 0.01$) in the number of MSCs growing on the scaffolds over 7 days and using BrdU incorporation demonstrated that MSCs proliferated on the scaffolds. Real-time PCR confirmed the temporally relevant expression of the osteogenic markers alkaline phosphatase, Cbfa-1, type-I collagen, osteocalcin, osteonectin and osteopontin by MSCs cultured on A-W scaffolds compared to MSCs grown on tissue culture plastic. An enzyme specific assay showed significantly higher ($p < 0.01$) alkaline phosphatase activity at days 7 and 14 compared to a commercially available calcium phosphate scaffold. Bespoke A-W scaffolds were engineered with a central channel for dynamic culture to increase cell penetration. Optimal seeding densities were determined using SEM and confocal microscopy was used to demonstrate that MSCs retained viability for 21 days culture and cell in-growth was facilitated. These results indicate good biocompatibility of A-W scaffolds and their potential as a bone replacement material.

(108) Development of Biocompatible, Biodegradable Electrospun Scaffolds for Tissue Engineering of Human Skin

Blackwood K., Freeman C.O., McKean R., Farthing P., Haycock J.W., Ryan A.J., Brook I., MacNeil S.

Departments of Engineering Materials, Chemistry and The Dental School, University of Sheffield, United Kingdom

Electrospun scaffolds show potential in becoming a synthetic substitute of dermis for skin tissue engineering. We report on the *in vitro* and *in vivo* degradation rates and biocompatibility of two lactide based electrospun scaffolds: poly-L-lactide (PLLA) and P(D,L)LA-co-polyglycolic acid (PGA) (50%, 75%, 85% PLLA by weight). Degradation *in vitro* was studied by light microscopy and SEM after incubating samples in Ringers solution at 37°C for up to 52 days. Degradation and biocompatibility *in vivo* was studied by implanting scaffolds subcutaneously into the flanks of adult rats for up to six months. Scaffolds were removed, processed by routine histological methods and examined by light microscopy and SEM. PLLA showed no evidence of degradation *in vitro* after 52 days and scaffolds implanted *in vivo* were clearly visible after 6 months. Implanted scaffolds were also associated with the formation of foreign body giant cells and were infiltrated by fibroblast and endothelial cells after 4 weeks. Granulation tissue was also observed with some scar formation. In contrast, P(D,L)LA-co-PGA 50% scaffolds underwent complete degradation *in vitro* by 20 days, whereas P(D,L)LA-co-PGA 85% scaffold breakdown was slower. When 85% P(D,L)LA-co-PGA scaffolds were implanted, a foreign body giant cell response was observed together with the formation of granulation tissue, but importantly this response diminished in line with a reduction of scaffold fibre diameter, with complete degradation seen after 5 months. In conclusion P(D,L)LA-co-PGA scaffolds show potential for skin tissue engineering as they are associated with cellular penetration, granulation tissue, limited scarring and have a controllable degradation rate.

(109) Development of Bioengineered Oral Mucosa by Tissue Engineering

Garzon I., Sanchez-Quevedo M.C., Gonzalez-Andrades M., Sanchez D., Labrot I., Alaminos M., Crespo P.V., Campos A.

Department of Histology (Group of Tissue Engineering), University of Granada, Spain. University Hospitals San Cecilio and Virgen de las Nieves, Granada, Spain

Introduction: Reconstruction of large oral mucosa defects is often challenging, since the shortage of healthy oral mucosa to replace the excised tissues is very common. In this work, we developed a new model of oral mucosa generated by tissue engineering using fibrin-agarose scaffolds.

Materials and Methods: Primary cell cultures of epithelial keratinocytes and stromal fibroblasts were generated by enzymatic treatment of normal oral mucosa biopsies. A substitute of the stroma was constructed using fibrin-agarose scaffolds with oral fibroblasts within. Finally, oral keratinocytes were seeded on the surface of the stroma substitute using air-liquid culture technique to ensure a proper differentiation of the epithelium. Histological analysis was carried out by optic and electron microscopy. Immunohistochemical and microarray studies were performed to evaluate PCNA expression in controls and bioengineered oral mucosa.

Results and Discussion: Histological analysis of the artificial tissues showed high similarities with normal oral mucosa controls. The epithelium of the oral substitutes had several layers, with desmosomes and apical microvilli and microplicae, whereas the artificial stroma allowed the fibroblasts to grow and proliferate

within. Both microarray and immunohistochemical studies demonstrated PCNA expression in the oral constructs, suggesting a proliferative activity in both the epithelium and the stroma. These results suggest that our model of oral mucosa shows several similarities with native human oral mucosa and could eventually be used for clinical purposes.

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(110) Development of Biofunctional Scaffolds for Bone Tissue Engineering

Röker S., Diederichs S., Korzhikov V., Tennikova T., Kasper C.

Institut für Technische Chemie, Universität Hannover, Callinstr. 3, 30167 Hannover, Germany

Institute of Macromolecular Compounds, Russian Academy of Sciences, Saint-Petersburg, Russia

Autologous bone tissue generation for transplantation is a very promising technique in orthopedic surgery and biomedical engineering, since it can eliminate problems of graft scarcity, immune rejection and pathogen transfer.

In order to apply this auspicious method, it is necessary to develop scaffolds with multifunctional properties mimicking the features of the extracellular matrix (ECM). The used material forms the desired structure and should provide all the necessary signals for the cells to proliferate, differentiate and interact.

With the aim of achieving these different applications, it is useful to modify a biocompatible matrix with different bioactive ligands (e.g. BMP-2, RGD peptides) enhancing cell adhesion, cell proliferation and differentiation.

The immobilisation of the ligands can be realised in two ways: either via direct adsorption to the matrix or via covalent bonding to "spacer arms", which are adsorbed on the matrix. With this second method the bioactivity of the ligands will not be reduced. This effect is known to occur within the direct adsorption process.

In this study, (co)polymers of the vinylsaccharide N-methacrylamidoglucose (MAG) were used as "spacer arms", because they already have been intensively studied concerning their physical properties and their applicability for the drug delivery. The applied matrix material is Sponceram[®], a macroporous ceramic doped with ZrO₂.

With regard to the application of the scaffolds in bone tissue engineering, the modified composite materials were tested in static and dynamic cell culture concerning cytotoxicity, cell viability/proliferation and osteogenic differentiation status using osteoblast precursor cells as a model cell line.

(111) Development of Biologic Filler for Soft Tissue Augmentation Using Human Fibroblasts and Placenta Extract

Oh E.J., Lim H.J., Choi J.H., Cho B.C., Chung H.Y.

Dept. of Advanced Organic Materials Science and Engineering, Kyungpook National University, Daegu, 702-701, Republic of Korea

Dept. of Plastic and Reconstructive Surgery, School of Medicine, Kyungpook National University, Daegu, 700-721, Republic of Korea

Purpose: In this study, biologic filler containing human fibroblasts for soft tissue augmentation was developed as a concept of cell therapy. The placenta extract, which has many bioactivities, was applied for accentuation of cell function. *In vivo* study was conducted with animal models to confirm the efficiency of biologic filler using human fibroblasts and placenta extract.

Materials and Methods: Forty nude mice were divided into four groups. Biologic filler containing human fibroblasts untreated (Group I), cultured with 0.1% placenta extract (Group II), 10% fetal bovine serum (FBS) (Group III), and both 0.1% placenta extract and 10% FBS (Group IV) was used in each group. Cultured human fibroblasts were injected into the back of each mouse with fibrin glue. These groups were compared over an 8-week period. The gross, histologic, and biomolecular studies were proceeded.

Results: In terms of geometric maintenance, volumes were 1.6 (Group II), 1.2 (Group III) and 1.9 times (Group IV) more reserved than one in untreated group (Group I) at 8 weeks respectively. Histologically, abundant proliferation of fibroblasts as well as extracellular matrices was visualized in experiment groups. Analyses of collagen, GAG, and mRNA expression of COL1A1 demonstrated significant differences between control (Group I) and experiment groups. There is no significant statistical difference between group II and III, on the other hand, group IV has statistically the best outcome among experimental groups.

Conclusion: The results imply the promising possibility of biologic filler material for soft tissue augmentation using human fibroblasts and placenta extract in clinical field.

(112) Development of Novel Micro and Nanopatterned Constructs for Tissue Engineering

Meredith D.O., Seunarine K., Gadegaard N., Wilkinson C.D.W., Riehle M.O.

Centre for Cell Engineering, University of Glasgow, Glasgow G12 8QQ, Scotland, United Kingdom

Tubular tissue engineering constructs offer great versatility for numerous biological applications. Current constructs employ traditional fabrication methodologies with limited scope for the pre-determination of the internal scaffold. The introduction of ordered micro and nanopatterns would be beneficial to the tailoring of a construct, providing indelible surface cues. Utilising a combination of lithographic techniques, polymer pressing and mechanical rolling, we have developed a flexible and repeatable methodology for producing ordered micro and nanopatterns within a multi-layer biodegradable polymer tube. A film of polycaprylactone (PCL) (50–100 µm) was melt-molded between nano and microtopography polydimethylsiloxane stamps. The PCL film was rolled (custom apparatus) into the multilayer tube and sealed with biogluce (or a suture thread)—this produced a uniform diameter lumen and micrometric spacers provide consistent spacing between the structure layers for cell penetration and colonisation. Constructs were

seeded with human fibroblasts and cultured for up to 120 hrs. Cell viability and adhesion were confirmed by the presence of multiple layers of cells in the structure. In this ‘first-step’, we introduce a totally novel method to fabricate tissue engineering constructs where at each interface the cell will be in contact with an intentional and carefully engineered surface. The method is versatile in allowing the interchanging of the surface nano and microtopography and interior dimensions. We aim to produce a vascular prosthesis and are currently tailoring the construct topography to enhance endothelial retention within the interior lumen and are moving the seeded construct into a bioreactor environment for long term culture.

(113) Development of Organotypic Skin Models to Investigate the Healing Properties of Oral and Dermal Fibroblasts

Kazmi B., Bhadal N., Wall I., Mudera V., Lewis M.P.

UCL, Eastman Dental Institute, Dept Biomaterials and Tissue Engineering, King's Cross, WC1X 8LD, United Kingdom

Oral wounds heal more quickly and with less obvious scarring than dermal wounds. A major component of that response lies with phenotypic differences in responses of the fibroblasts that make up the tissues. Harnessing this preferential oral healing phenotype for extra-oral application could improve the performance of bioengineered organotypic tissue substitutes, which are increasingly being used for wound healing.

Our aims were to study whether the phenotypic differences that exist between human oral fibroblasts (HOF) and human dermal fibroblasts (HDF) have differential effects on the response of 3D organotypic models. The initial phase was to optimise the organotypic models and to challenge them, through wounding, and study cellular responses by using analysis of mRNA expression.

Here we use patient matched HOF and HDF seeded into a type I collagen matrix, with an overlay of immortalised or normal human keratinocytes, to construct models of oral mucosa and skin. Investigating different organotypic culture systems determined that models created inside culture inserts made subsequent experimentation easier.

Initial data showed that in co-culture cells behaved as expected and morphologically both bioengineered tissues appeared similar. HOF contracted collagen matrices more than HDF, suggesting HOF are more efficient at remodelling the extracellular matrix environment *in vitro*, regardless of keratinocyte cell type. Correlating to this, models of oral mucosa expressed higher levels of mRNA for α -smooth muscle actin than skin models, re-emphasising a comparatively more ‘activated’ state before and after wounding. Our findings suggest that heterotypic models containing oral fibroblasts could be used in extra-oral environments.

(114) Development of Polymer/Ceramic Nanocomposites for Soft Tissue Regeneration and Replacement

El Fray M., Zdebiak P., Piegat A.

Szczecin University of Technology, Polymer Institute, Division of Biomaterials and Microbiological Technologies, Pulaskiego 10, 70-322 Szczecin, Poland

Soft tissue regeneration and replacement is a great challenge for development of new materials, with unique mechanical and biological properties. Especially nanometric constructs, containing inorganic (ceramic) fillers in different polymer matrices are becoming an emerging type of biomaterials for such applications. Biodegradable polymer matrices can be of natural or synthetic origin. In case of synthetic materials, especially those derived from monomers from renewable resources are becoming very important, therefore we proposed to use fatty acids as excellent monomers, especially for elastomeric polymer matrices. Due to combination of elastomeric matrices with nanometer-size ceramic particles, various interesting materials can be prepared. Among the available elastomeric matrices, we used poly(aliphatic/aromatic-ester)s (PED), composed of semicrystalline poly(ethylene terephthalate) (PET) or poly(butylene terephthalate) (PBT) hard segments and amorphous dilinoleic acid (DLA) and/or poly(ethylene glycol) (PEG) soft segment sequences. PET/DLA and PBT/DLA/PEG copolymers with weight ratio 30/70 were obtained by *in-situ* polycondensation in the presence of different concentrations of TiO₂ nanoparticles or nanocrystalline hydroxyapatite (HAP). Special preparation of monomers prior the *in situ* polymerization has resulted in remarkable improvement of mechanical properties induced by the presence of nanofillers. This feature is especially important for these applications where material is subjected to oscillatory deformation such as in metacarpophalangeal joints or heart patches. In case of PEG soft segments, it has also been found that HAP can simultaneously act as reinforcing and stabilizing agent. Simultaneously, we also found that TiO₂ nanoparticles can act as agent regulating degradation processes.

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(115) Development of the Regenerative Vascular Graft Having an *In Vivo* Repopulationality

Terada D., Sawada K., Ogata H., Ehashi T., Hiraku K., Kamata W., Yoshida K., Funamoto S., Nagaya N., Kishida A., Fujisato T., Nakatani T.

Osaka institute of Technology, Dept Biomed Eng, Osaka, Japan

Although an artificial blood vessel is in general use, the development of regenerative vascular grafts is strongly desired especially for the pediatric patients. In this study, regenerative collagenic vascular grafts were developed from porcine aorta by removing cells and structural proteins except collagen from the tissue.

Porcine aorta was isolated from the Claw mini-pig (Japan Farm, Co. Ltd.). The tissue was placed in a vacuum oven at 120°C to cross-link collagen fibers. Elastin fibers were then taken away from the tissue by enzymatic digestion using elastase of 0.56 u/ml in tris buffer solution at 37°C with gentle stir. The tissues were incubated in 80% ethanol solution for 3 days at 37°C to remove phospholipids from the inside. The obtained tissues were subjected to histological and biomechanical studies. The vascular grafts made of mini-pig descending aorta were transplanted allogeneically.

There was no thrombus on the intimal surface and aneurysm formation even after 3 months of the implantation. A large amount of the cell migration into the graft was observed. These cells were

identified immunohistologically as smooth muscle cells and fibroblasts. And no calcific deposition was seen in the explanted graft after 3 months of the implantation. Currently long-term implantation experiments, 6 and 12 M, are in progress.

The processed graft may have better ability to promote cell infiltration and tissue remodeling compared with the acellular tissue without elastin digestion. We conclude that the collagenic vascular graft developed in this study may be adapted to the vascular tissue regeneration.

(116) Development of Tissue Engineered Blood Vessels with a Functional Medial Muscle Layer

Yazdani S.K.^{1,2}, Jarajapu Y.P.R.², Machingal M.^{1,2}, Watts B.², Van Dyke M.E.², Christ G.J.²

¹Wake Forest University, Department of Biomedical Engineering, Winston-Salem, NC, United States of America

²Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, United States of America

Development of tissue engineered vascular constructs that contain a physiologically relevant/functional muscularized vessel wall (comparable to native vessels) is highly desirable, but has been challenging. The goals of this project were to increase the attachment of vascular smooth muscle cells (VSMCs) to decellularized scaffolds *in vitro*, and to accelerate the tissue-cell maturation/formation process through bioreactor preconditioning.

Primary cultures of rat aortic vascular smooth muscle cells (VSMCs) were harvested and statically seeded (50 million cells/ml) on acellular porcine carotid arterial segments in the absence and presence of the adventitial layer. Following seeding, cells were either maintained under static conditions or subjected to cyclic strain within a bioreactor system. H & E and DAPI staining were used to determine the impact of scaffold surface modification (i.e., adventitia), cell seeding density and bioreactor preconditioning on cell proliferation and organization.

Removal of the adventitia had dramatic effects on VSMC attachment and retention on the decellularized scaffold. Induction of 2 weeks of cyclic strain within a bioreactor system (pulse rate of 60 beats/min, pressure range of 80–120 mmHg) further enhanced cellular proliferation and maturation. This study indicates that VSMCs seeded on decellularized porcine carotid arteries adhere, proliferate, and re-orient under cyclic strain conditions. We have determined proper seeding methods and structural modifications required for improved static seeding of VSMCs. The apparent benefits of removing the adventitial layer are promising, but further investigation is required to determine the specific scaffold surface proteins responsible for these effects and to further refine the bioreactor protocols and media composition.

(117) Development of Tissue Engineered Skin for the Treatment of Severe Burns

Cortiella J., McClure M.L., Niles J.A., Mlcak R.P., Warner S., Bynum S., Naylor K.J., Fung C.W., Lee J., Nichols J.E.

University of Texas Medical Branch at Galveston, United States of America

We feel that to support the growth and regeneration of tissues after severe skin injury a suitable support nano-scaffold and treatment delivery system must be designed. The scaffold must: (1) support cell chemotaxis, proliferation and tissue growth; (2) provide a suitable substrate for adherence; (3) support production of extracellular matrix; (4) resorb from the wound site; (4) reduce water loss and help maintain homeostasis immediately after injury. We feel the scaffold must also be able to be applied to a wound of any size or shape. With current treatments the production of a new dermal component is usually unsatisfactory, leaving the patients severely scarred. We have been evaluating the ability of embryonic (ES) or adult stem (AS) cells to develop into any or all components of dermal tissue. ES or AS cells were added to a biodegradable matrix that functioned as a scaffolding for the cells and were incubated with both epidermal growth factor and fibroblastic growth factor for two days prior to implantation in an murine injury model. This allowed the cells to differentiate. Mice were exposed to 2-Chloroethy-ethyl sulfide (CEES or half mustard). The site of the chemical exposure was then surgically removed after exposures were completed. A 5mm piece of growth factor primed cell/matrix construct was then placed onto the wound site. Data suggests that the stem cells differentiated in culture, decreased wound-healing time, and stimulated the production of new dermal components in the injured animals.

(118) Differential Effects of Gel-Like Carrier Materials on *In Vitro* Chondrogenesis of Adipose Tissue-Derived Stem Cells and Ectopic Cartilage Stability *In Vivo*

Dickhut A., Gottwald E., Heisel C., Richter W.

Division of Experimental Orthopaedics, Orthopaedic University Hospital, Heidelberg, Germany

Introduction: Adipose tissue-derived stem cells (ATSC) are discussed as an attractive alternative cell source for therapy of focal cartilage defects and gel-like carrier materials become more and more important in recent cartilage repair procedures. The aim of this study was to investigate whether such materials support the *in vitro* chondrogenesis of ATSC and may positively influence their ectopic cartilage formation capacity.

Materials and methods: ATSC were cultured as high-density pellets or embedded in collagen-type-I-gel, fibrin glue, Matrigel and PuraMatrix peptide hydrogel and kept under chondrogenic conditions for up to six weeks. Chondrogenesis was evaluated based on histology, quantitative gene expression analysis, proteoglycan content and collagen synthesis. After five weeks of chondrogenesis, carrier constructs and carrier-free pellets were transplanted subcutaneously into SCID mice. Four weeks later explants were analysed by histology.

Results: Chondrogenesis was supported by all materials, but differential effects were seen for collagen type II and X gene expression levels, collagen synthesis and proteoglycan deposition. Collagen synthesis was enhanced by fibrin and Matrigel, while proteoglycan deposition and *in vitro* chondrogenesis were improved in PuraMatrix. Only Matrigel did not delay induction of COL2A1 mRNA expression and was able to reduce the undesired ALP-

activity, ALP gene expression levels and calcification of ectopic transplants *in vivo*.

Conclusion: The results demonstrate that gel-like biomaterials offer the opportunity to influence chondrogenic differentiation and *in vivo* stability of MSC-derived transplants. Beyond the here shown superior performance of PuraMatrix and Matrigel, their integration behaviour and durability should further be analysed in a cartilage defect model.

(119) Differentiation and Proliferation of Human Adipose Tissue-derived Mesenchymal Stem Cells Under Hypoxic Conditions

Fiedler T.¹, Salamon A.¹, Adam S.¹, Rychly J.², Peters K.¹

¹Junior Research Group "Bone Regeneration", Department of Cell Biology, Biomedical Research Centre, Schillingallee 69, 18057 Rostock, Germany

²Department of Cell Biology, Biomedical Research Centre, Schillingallee 69, 18057 Rostock, Germany

Adipose tissue is a promising source for mesenchymal stem cells. Liposuction is a minimally invasive surgery and a large number of stem cells can be isolated from the stromal vascular fraction (SVF) of liposuction material. Thus, adipose tissue might have the potential to outmatch bone marrow as a stem cell source in regenerative medicine. The SVF of lipoaspirates can be separated in a CD34-positive and a CD34-negative fraction. Both cell types have the capacity to undergo adipogenic and osteogenic differentiation *in vitro* in response to the respective stimuli, but knowledge about the CD34 subpopulations is incomplete.

Low oxygen partial pressure (hypoxia) is a regular physiological state in wound healing regions. Therefore, we analyzed the proliferation and differentiation of both unstimulated and osteogenically stimulated CD34-positive and -negative cells under hypoxia (2% O₂) and treatment with the hypoxia-mimicking agent cobalt chloride (CoCl₂), compared to cells grown under atmospheric ("normoxic") oxygen partial pressure.

We found that CD34-negative cells exhibit a higher degree of osteogenic differentiation than CD34-positive cells. Differentiation is decreased by hypoxia and CoCl₂-treatment during osteogenic stimulation compared to the normoxic controls in both CD34 subpopulations, while proliferation is induced by hypoxia and reduced by CoCl₂. Thus, controversial results were obtained for the CD34 subpopulations and also for hypoxia and CoCl₂ experiments. Studies on variations in oxygen partial pressure can contribute to elucidate the signalling of mesenchymal stem cells in wound healing *in vivo*.

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(120) Differentiation Capacity of Processed Lipoaspirates Before and After Cryopreservation

Dickens S., Vermeulen P., Hendrickx B., Van den Berge S., Vranckx J.J.

Laboratory of Plastic Surgery and Tissue Engineering Research, Department of Plastic & Reconstructive Surgery, KULeuven University Hospital, Katholieke Universiteit Leuven, B-3000, Belgium

Therapeutically, multilineage stem cells (SC) show great potential for applications in tissue engineering research and gene therapy. Human processed lipoaspirates (PLA) are a source of multiple adult SC, next to bone-marrow (BM)SC that are able to differentiate into different mesenchymal cell lineages. PLA are easier to obtain, with less patient discomfort and in more abundant cell numbers and with the same differentiation capacity as BMSC. Cryopreservation technology has already been used successfully for long term preservation of skin grafts in plastic surgery. So next to the use of freshly isolated PLA, long term preservation of this autologous cell type might have beneficial potential in future application in clinics.

We isolated PLA and grew them at 37°C and 5% CO₂. PLA were also frozen and stored for a few weeks in DMSO containing medium under liquid nitrogen conditions. PLA (fresh and frozen) were analysed for cell morphology and differentiation capacity toward adipogenic, chondrogenic and osteogenic lineages by histology staining (Oil Red O, Alcian Blue and Alizarin Red). Also the difference in proliferation between both cell populations was investigated by MTT assays.

PLA are capable of proliferation and differentiation after cryopreservation but their proliferation and induction capacity toward mesenchymal lineages is diminished by freeze-thawing cryopreservation. Nevertheless frozen PLA cells show similar morphology.

In conclusion, frozen PLA can be used after thawing, but fresh PLA are more optimal to be used in biological or clinical research.

(121) Differentiation of Adipose-Derived Stem Cells to a Schwann Cell Phenotype

Kingham P.J.¹, Kalbermatten D.F.¹, Mahay D.¹, Armstrong S.J.¹, Wiberg M.², Terenghi G.¹

¹Blond McIndoe Research Laboratories, University of Manchester, United Kingdom

²Department of Surgical & Perioperative Science, University Hospital, Umea, Sweden

Schwann cell transplantation has been shown to enhance experimental peripheral nerve repair. The clinical application of this therapy is however limited by donor site morbidity and the inability to generate a sufficient number of cells quickly. As an alternative, we have investigated whether adult stem cells, isolated from adipose tissue, can be differentiated into functional Schwann cells. Rat visceral fat was enzymatically digested to yield rapidly proliferating fibroblast-like cells which expressed stem and progenitor cell markers including *stro-1* and *nestin*. When the cells were treated with a mixture of glial growth factors (GGF-2, bFGF, PDGF and forskolin) they assumed a spindle shaped morphology similar to Schwann cells. Immunocytochemical staining indicated that approximately 40% of these cells expressed the glial markers, S100 and GFAP, indicative of differentiation. The function of these cells was then assessed by co-culture with NG108-15 motor neuron-like cells. Differentiated stem cells significantly ($p < 0.05$ by one-way ANOVA) enhanced the number of NG108-15 cells expressing neurites, the number of neurites per cell and the mean

length of the longest neurite extended. These results indicate that adipose tissue contains a pool of regenerative cells which can be differentiated to a functional Schwann cell phenotype. More recently, we have identified a sub-population of adipose tissue cells which express the receptor tyrosine kinase, ErbB3, a protein required for the development of Schwann cells from the neural crest. We hypothesize that isolation and expansion of this cell population will enhance the therapeutic potential of adipose-derived stem cells for peripheral nerve repair.

(122) Differentiation of Human Bone Marrow Stem Cells within RGD Functionalised, Proteolytically Sensitive PEG Gels

Gurav N.¹, Lutolf M.P.², Raeber G.P.², Hubbell J.A.², Di Silvio L.³

¹Northwick Park Institute of Medical Research (NPIMR), Northwick Park Hospital & St Marks Trust (Y Block), Watford Road, Harrow, HA1 3UJ, United Kingdom

²Institute for Biomedical Engineering, ETH and University of Zurich, Laboratory of Stem Cell Bioengineering, Switzerland

³The Guy's, King's and St Thomas' Hospital Medical and Dental Institute, King's College London, Biomaterials Science, Guy's Campus, St Thomas' Street, London, SE1 9RT, United Kingdom

A PEG-based hydrogel system (1), functionalised with RGD peptides to provide cell adhesion, and cross-linked via matrix metalloprotease (MMP)-sensitive peptides, was evaluated as a carrier for human BMSCs. An aqueous solution of vinylsulfone-functionalized PEG was reacted with Cysteine-containing RGD peptide; cross-linking occurred in a few minutes by reaction of this precursor solution with a solution containing a MMP-sensitive peptide (GCRD-GPQGIWGQ-DRCG) pre-mixed with stem cells. Osteoblastic differentiation was induced by 50 µM ascorbate-sulphate, 100 mM β-Glycerol phosphate, and 100 nM of dexamethasone and chondrocytic differentiation by 10 ng/ml of TGF-β1.

Results show that the gel allowed even dispersal of cells which remained viable and metabolically active for up to 28 days. Cells were able to differentiate towards specific lineages with osteogenic differentiation identified by alkaline phosphatase (ALP) activity and mineralized nodules confirmed with von Kossa staining. Chondrogenic differentiation was confirmed by positive histological staining for Chondroitin 4 Sulphate, Chondroitin 6 Sulphate, Keratin Sulphate, Biglycan and Decorin.

Our results demonstrate that the synthetic PEG-based, 3-D gel system can be used as a carrier for stem cells, allowing cell-mediated proteolytic migration and remodelling during MSC differentiation into different lineages. Under appropriate conditions cells respond to signalling cues from the environment and become committed osteoprogenitor cells and chondrocytic cells. This system which is injectable has potential for a wealth of applications in tissue engineering for repairing small bone and cartilage defects.

Reference

1. Lutolf MP, Hubbell JA. 2003, Synthesis and physicochemical characterization of end-linked poly(ethylene glycol)-co-peptide hydrogels formed by Michael-type addition. *Biomacromolecules* 4(3):713–22.

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(123) Differentiation Potential of Primitive Cells Isolated from Immature Foreign Body Reaction

Vranken I., Lebacqz A., De Visscher G., Flameng W.

Katholic University of Leuven, Belgium

Implanted non-degradable materials are subjected to a foreign body reaction (FBR). The initial mechanism comprises the subsequent steps of host fibrinogen adhesion, denaturation and macrophage adhesion. After approximately 2 weeks, the mature FBR capsule consists of an internal layer of macrophages, several layers of (myo)fibroblasts and an external layer of mesothelial cells. The conversion of the initial reaction into the mature capsule is not completely studied, but recently we have observed that a significant number of CD117+, CD34+ or CD271+ primitive cells were attracted very early in the FBR development.

In this rat study we examined the clonal and differentiation potential of different primitive cells from intraperitoneal implants. Cells were isolated from 3-day implants followed by two-step magnetic labeling and separation. Culturing in appropriate media showed several hematopoietic and mesenchymal colonies in the lin-CD117+, lin-CD34+ and lin-CD271+ cell fractions. The lin-CD271+ primitive mesenchymal cells were stimulated to adipogenic and osteogenic differentiation. An Oil Red O and BCIP/BCT substrate staining, respectively, confirmed the differentiation. After stimulation into myofibroblastic differentiation, stainings for vimentin, alpha smooth muscle actin, smoothelin and desmin as well as a gene expression study confirmed their differentiation into myofibroblasts.

The current study confirms the presence of stem/progenitor cells during early FBR by showing clear primitive cell properties such as clonality and differentiation potential. The relevance of these findings to tissue engineering is twofold: a better understanding of the reaction to implants and a potential use of these cells in both *in vivo* or *in vitro* tissue engineering.

(124) Differentiation Property of Mesenchymal Stem Cells Isolated by Ligand-Immobilized Column System

Mahara A., Yamaoka T.

National Cardiovascular Center Research Institute, Advanced Medical Engineering Center, Department of Biomedical Engineering, Suita, Osaka, Japan

Mesenchymal stem cells (MSCs) have multipotent and proliferative activity. In many cases, MSCs were separated by FACS system based on the presence of the specific surface marker. However, the density of surface marker is well known to be dramatically changed with developmental stage and its differentiation property. In this study, we focused on its density for preparing the highly purified MSCs. To isolate an MSC population which has homogenous density of the surface marker, the novel ligand-immobilized column systems were designed and established. The injected cells rolled on the inner surface under a medium flow condition like a rolling adhesion phenomenon of the leukocytes in the blood vessel.

AntiCD34 antibody was immobilized on the flat surface of the column at the density of 200 µg/m². Roughly purified MSCs were prepared from a bone marrow of C57BL/6 mouse by the conventional method. Only when the MSCs were injected into the

antibody-immobilized column, delayed fractions were observed, suggested that the MSCs were separated by the marker-antibody specific rolling mechanism. Next, the purified fractions were incubated with the osteoblastic differentiation medium for 4 days. In this condition, the expression of specific genes related to the differentiation was increased 10 to 5-fold compared with unpurified MSCs. These results suggested that MSCs were purified using the column in about 10 min and the purified MSC fraction was composed of the pure population with a high potential for the differentiation.

(125) Donor Age Has a Crucial Impact on MSC Number, Function and Protein Expression Pattern

Kasper G., Mao L., Geißler S., Trippens J., Kaspar K., Perka C., Duda G.N., Klose J.

Musculoskeletal Research Center Berlin, Charite—Universitätsmedizin Berlin, Germany
Department for Human Genetics, Charite—Universitätsmedizin Berlin, Germany

Progenitor cells, such as mesenchymal stem cells (MSCs), have the potential to be used therapeutically to augment physiological regeneration processes. This study correlated functional to proteomic changes in MSCs derived from young and old male Sprague Dawley rats.

MSC populations from young and old donors were homogeneous in morphology and similar in their expression levels of surface marker proteins. However, the cell yield as well as the number and portion of AP-positive colony forming units decreased with age. Furthermore, the migratory capacity of MSCs decreased in elderly donors and the cells seemed to harbour a lower differentiation potential.

The proteome of low passage MSCs from young and old donors was analysed by high resolution 2D electrophoresis under conditions of *in vitro* culturing as well as after the application of an osteogenic stimulus. In total, 34 (w/o osteogenic stimulus) and 64 proteins (under osteogenic stimulus) were detected as age-dependently expressed, among which 14 proteins were common under both conditions. Shared molecular functions altered under both conditions were associated with cytoskeleton organization and antioxidant defence. The later alteration might be causative for the increased number of senescent (β-galactosidase positive) cells observed in high passage cultures from older animals.

In summary, number and functionality of MSCs seem to be altered with age, which through the regulation of MMPs also seem to modify the microenvironment. Our observation indicates that the calponin protein family, galectin-3 and MMP-9 proteins, which are known to be associated with bone physiology, might be relevant to MSC ageing.

(126) Donor Variation and Loss of Multipotency During In Vitro Expansion of Human Mesenchymal Stem Cells for Bone Tissue Engineering

Siddappa R., Licht R., Van Blitterswijk C., De Boer J.

Department of Tissue Regeneration, Institute for BioMedical Technology, University of Twente, Enschede. P.O. Box 217, 7500AE, The Netherlands

The use of multipotent human mesenchymal stem cells (hMSCs) for tissue engineering has been a subject of extensive research. The donor variation in growth, differentiation and *in vivo* bone forming ability of hMSCs is a bottleneck for standardization of therapeutic protocols. In this study, we isolated and characterized hMSCs from 19 independent donors, aged between 27 and 85 years, and investigated the extent of heterogeneity of the cells and the extent to which hMSCs can be expanded without losing multipotency. Dexamethasone-induced ALP expression varied between 1.2 and 3.7-fold but no correlation was found with age, gender or source of isolation. The cells from donors with a higher percentage of ALP positive cells in control and dexamethasone-induced groups showed more calcium deposition than cells with lower percentage of ALP positive cells. Despite the variability in osteogenic gene expression among the donors tested, ALP, Collagen type1, osteocalcin and S100A4 showed similar trends during the course of osteogenic differentiation. *In vitro* expansion studies showed that hMSCs can be effectively expanded up to 4 passages (approximately 10–12 population doublings from a P0 culture) while retaining their multipotency. Our *in vivo* studies suggest a correlation between *in vitro* ALP expression and *in vivo* bone formation. In conclusion, irrespective of age, gender and source of isolation, cells from all donors showed osteogenic potential. The variability in ALP expression appears to be a result of sampling method and cellular heterogeneity among the donor population.

(127) Dynamic Compression Counteracts IL-1 β Induced iNOS and COX-2 Expression in Chondrocytes Cultured in Agarose Constructs

Chowdhury T.T.¹, Akanji O.O.¹, Arghandawi¹, Salter D.M.², Bader D.L.¹, Lee D.A.¹

¹School of Engineering and Materials Science, Queen Mary University of London, Mile End Road, London, E1 4NS, United Kingdom

²Queens Medical Research Institute, 47 Little France Crescent, Edinburgh University, Edinburgh, EH16 4TJ, United Kingdom

Mechanical load will influence chondrocyte mechanotransduction processes such that inappropriate signalling will lead to the breakdown of articular cartilage. Clarification of the specific signalling mechanisms is thus vital for the development of tissue engineered strategies for cartilage repair. Using the chondrocyte/agarose model in conjunction with a custom designed bioreactor, the present study examines the effects of IL-1 β and dynamic compression on the activation of the pro-inflammatory genes, namely inducible nitric oxide (iNOS) and cyclo-oxygenase-2 (COX-2).

Real-time quantitative PCR analysis revealed that the presence of IL-1 β enhanced iNOS and COX-2 mRNA levels in a temporal manner, with peak expression at 6 or 12 hours, followed by a rapid decline after 48 hours. The application of 15% dynamic compressive strain at 1 Hz frequency reversed the IL-1 β induced expression of iNOS and COX-2 at all time points investigated. In addition, the response to IL-1 β and dynamic compression was abolished by the presence of an inhibitor for the p38 mitogen activated protein kinase (MAPK) pathway (SB203580).

In summary, IL-1 β will stimulate the expression of iNOS and COX-2 mRNA levels via upstream activation of p38 MAPK. The response could be reversed with the application of dynamic compression, suggesting interactive intracellular signalling pathways

by IL-1 β and mechanical load. The current study will help to provide a better understanding of the processes which regulate chondrocytes and will yield new insights on cell or tissue based therapies for cartilage tissue engineering.

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(128) Dynamic Mesenchymal Stem Cell Culturing for Bone Tissue Engineering

Diederichs S., Riechers D., Sempf F., Kasper C., Scheper T., Van Griensven M.

Institut für Technische Chemie, Universität Hannover, Callinstrasse 3, D-30167 Hannover, Germany

Static cell culture with the aim of tissue engineering applications has been proven to be disadvantageous in several ways. On the one hand, it has been shown that constructs grown in static cell culture lack the mechanical stability native tissue exhibits. Mechanical stimulation has therefore become a substantial tool in tissue engineering to accustom cells to their future physically active environment. Moreover, differentiation of stem and progenitor cells has been proven to be influenced by mechanical strain.

In this work, the effect of mechanical strain on the differentiation of the preosteoblastic cell line MG-63 and adipose tissue derived mesenchymal stem cells was analysed. Therefore, cells were subjected to a cyclic strain on a flexible silicone substrate. Cell viability (MTT assay) was not affected by the applied strain, while osteogenic markers showed different expression rates (RT-PCR). Moreover, an electric cell-substrate impedance sensing (ECIS) device for measurement during strain experiments was developed. This was utilized in order to determine morphological changes due to the applied load.

On the other hand, in static cell culture usually nutrient and oxygen supply as well as waste removal especially on 3D scaffolds are heterogeneous leading to reduced growth rates or even tissue death. Therefore, dynamic cell culturing using specifically customised bioreactors has become important for tissue engineering. In this work, the cultivation of the cells in a rotating bed system bioreactor was aspired in order to enhance the growth of a biologically active and reproducible tissue.

(129) Effect of α 2, α 5 and α v β 3 Integrins on Mesenchymal Stem Cell Adhesion to Extracellular Matrix Proteins

Dreger S.A., Taylor P.M., Yacoub M.H., Chester A.H.

Division of Cardiothoracic Surgery, NHLI, Imperial College London, Heart Science Centre, Harefield Hospital, Harefield, Middlesex, England, UB9 6JH, United Kingdom

Presence of specific extracellular matrix (ECM) proteins on scaffold material will govern the ability of cells to populate and remodel the scaffold. In this study, we have assessed the adhesion, morphology and cell viability of mesenchymal stem cells (MSCs) on various ECM protein substrates.

MSCs cultured from human bone marrow were serum starved and seeded (5×10^3 cells/well) onto 96-well plates coated with human vitronectin, human fibronectin or bovine type I collagen. At various time points, cell morphology, adhesion and viability (MTS

Assay) were assessed using CellTrace™ calcein green AM labelled cells. Blocking cellular adhesion was also demonstrated with the use of $\alpha 2$, $\alpha 5$ and $\alpha v \beta 3$ integrin antibodies.

After one hour incubation, $71.6 \pm 7.6\%$ viable cells adhered to fibronectin, $30.3 \pm 3.8\%$ to vitronectin and $44.5 \pm 8.1\%$ to collagen ($p < 0.05$ versus fibronectin). Maximal attachment was achieved by 24 hours: fibronectin ($83.3 \pm 18.8\%$) > vitronectin ($81.2 \pm 12.6\%$) > collagen ($62.7 \pm 20.1\%$). Cells were rounded on all three ECM substrates after 1 hour incubation and had begun to spread out and elongate after 8 hours (fibronectin) and 24 hours (vitronectin). However, cells on collagen were still rounded after 48 hours. Blocking cellular adhesion with a combination of the integrin antibodies ($\alpha 5 + \alpha 2$, $\alpha 5 + \alpha v \beta 3$, $\alpha 5 + \alpha 2 + \alpha v \beta 3$) significantly inhibited adhesion to fibronectin but not vitronectin.

These results suggest that integrins may synergistically function to enhance cell attachment as well as facilitate cell proliferation on ECM proteins. Therefore, incorporation of preferred ECM proteins into a biodegradable scaffold such as collagen should enhance the development of the tissue engineered construct.

(130) Effect of Crosslinking of CEM on Cell Attachment, Proliferation and Morphology

Burugapalli K., Chan J.C.Y., Kelly J.L., Pandit A.

National Centre for Biomedical Engineering Science, National University of Ireland, Galway, and Department of Plastic, Reconstructive and Hand Surgery, University College Hospital, Galway, Ireland

Intact extracellular matrix (ECM) based scaffolds have been used clinically in non-crosslinked or chemically crosslinked forms. It is hypothesised that varying degree of crosslinking would provide a means to tailor the properties of an intact ECM based scaffold. In this study, the effect of 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide (EDC) crosslinking on the ability of cholecyst-derived extracellular matrix (CEM) to support cells *in vitro* was investigated.

CEM was crosslinked with EDC and N-hydroxy succinimide in 50mM MES buffer (pH 5.5). Six different crosslinking concentrations ranging between 0.0001 and 0.01 mM of EDC per mg of CEM were used. Fibroblasts were cultured on the scaffolds for 1, 3, 5 and 7 days, in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin and 1.25 mg/L Amphotericin-B. The morphology of the cells on scaffolds was studied using DAPI and rhodamine phalloidin staining and scanning electron microscopy. Viable cell counts as a function of time were determined using AlamarBlue™ assay.

All scaffolds supported cell attachment and proliferation. Among the different scaffold variants, no significant differences were observed for the initial cell attachment as well as cell proliferation rate at each time point. The fibroblasts proliferated to near confluence by 7 days of culture and demonstrated a dendritic phenotype (1) on all scaffolds. It is concluded that crosslinking did not effect the *in vitro* attachment, viability, proliferation as well as morphology of fibroblasts on CEM.

Reference

1. Grinnell F, et al. Mol Biol Cell 2003;14(2):384-95.

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(131) Effect of Cryopreservation on Adherent Cells for Bone Tissue Engineering

Ma X.¹, Liu Y.¹, Ge D.¹, Cui Z.F.², Liu T.Q.¹

¹Dalian R & D Center for Stem Cell & Tissue Engineering, Dalian University of Technology, Dalian 116024, China

²Department of Engineering Science, Oxford University, Parks Road, Oxford OX1 3PJ, United Kingdom

The fast development of tissue engineering has made the long-term preservation of cells and engineered tissues necessary to ensure the off-the-shelf availability to clinicians and overcome the limitation of production cycle. Osteoblasts are considered as the seeding cells for tissue-engineered bone construction. In bone tissue engineering it is essential for cells to remain attached to the bone scaffold surface. The behaviors of adherent cells and cells in suspension are different during cryopreservation, so that the investigation of the influence of attachment on the cryopreservation process is of significance for the cryopreservation strategies for engineered tissues.

In this study, the different protocols were examined to investigate the response of adherent cells during CPAs loading. Osteoblast suspensions were seeded on the bottom surface of 24-well plate as a simulating model of the adherent cells. A vitrification cocktail of DMSO, formamide and 1,2-propanediol was loaded by one-step, multiple stepwise and continuous programs. The cell viabilities assayed by CCK-8 of adherent cells and cell suspension after loading of vitrification solution were compared.

The cell viability of adherent cells was significantly lower than that of the cell suspension after vitrification solution addition and dilution. The recovery of adherent cell decreasing with long equilibrium time indicated the high toxicity of the high concentration of CPAs. The results also showed that cells adherent to scaffold are much more sensitive to the solute injury and osmotic damage than the suspend ones.

(132) Effects of Electromagnetic Fields on Chondrocytes: An In Vitro Study

Kozaci L.D.¹, Bilgin M.D.², Ek R.O.³, Kafienah W.⁴

¹Adnan Menderes University, Faculty of Medicine, Dept. Biochemistry, Aydin 09100, Turkey

²Adnan Menderes University, Faculty of Medicine, Dept. Biophysics, Aydin 09100, Turkey

³Adnan Menderes University, Faculty of Medicine, Dept. Physiology, Aydin 09100, Turkey

⁴University of Bristol Academic Rheumatology, Avon Orthopaedic Centre, Southmead Hospital, Bristol BS10 5NB, United Kingdom

Cartilage tissue engineering involves isolating the patient's own chondrocytes or stem cells, expanding and differentiating them, seeding them onto a biomaterial scaffold. However, it is limited by the risk of cell dedifferentiation during proliferation. Techniques with negligible toxic effects are needed to minimize the risk of dedifferentiation and to stimulate cell expansion without increasing passage numbers. Articular chondrocytes are responsive to biophysical stimuli such as electromagnetic field (EMF), ultra-

sound, and mechanical stresses. EMF plays a regulatory role in cartilage metabolism by increasing the chondrocyte proliferation and synthesis of extracellular matrix components, and reducing the matrix degradation (1, 2).

Aim of this study was to achieve the required chondrocyte numbers without causing any toxicity and dedifferentiation, by reducing the passage numbers in cell cultures by using EMF.

The effects of EMF on chondrocytes, isolated from hyaline cartilage, were investigated in this study. Chondrocytes were isolated from nasal bovine cartilage and cells were cultured with/without growth factors (Insulin-like growth factor or fibroblast growth factor) for 7 days in 24-well plates. Pulsed or continuous EMF was also applied to the plates (30 min/day, 7 days). At the end of culture period, cells were counted. Expression levels of mRNA for type I and II collagens and aggrecan were determined by real-time PCR. In presence of growth factors, EMF exposure induced cell proliferation and matrix protein expression.

EMF may be used as a chondroprotective agent for the treatment of chronic diseases such as osteoarthritis.

References

1. Sollazzo V et al. *Electro Magnetobiol* 1996;15:75–83.
2. Fini M et al. *Journal of Orthopaedic Research* 2005; 23: 899–908.

(133) Effect of Ion Plasma Modification and Carbon Coating on Biocompatibility of PTFE

Remeeva E., Rozanova I., Elinson V., Sevastianov V.

Centre for Biomaterials Research, Research Institute for Transplantation and Artificial Organs, Shukinskaya 1, 123182 Moscow, Russian Federation

Polyethyleneterephthalate (PTFE) is well-known and commonly used material for vascular grafts, valves etc. Platelet adhesion, protein adsorption and fibroblast growth onto the PTFE surface modified by CF₄-ion plasma bombardment followed by carbon film deposition were investigated *in vitro*.

PTFE surface was modified using 30 min CF₄-ion plasma treatment. Then 10 and 100 nm thickness diamond-like carbon films (α -C:H) were deposited. The physicochemical properties of samples were analyzed by AFM and contact angle measurements. Human serum albumin (HSA) adsorption was estimated using spectrofluorimetry technique. Platelet adhesion was studied by SEM. Mouse NIH 3T3 fibroblasts were incubated with PTFE disks for 1, 3 and 6 days and then observed with SEM and fluorescent microscope.

It was shown that CF₄ plasma treatment increases the hydrophobic character and RMS roughness of PTFE, while subsequent α -C:H film deposition results in a more hydrophilic surface and rather small increase of R_q with respect to untreated one. Plasma treatment of PTFE appeared to lead to the growth of HSA adsorbed and amount of platelets adhered. Meanwhile carbon coated surface revealed evident decrease in protein adsorption and platelet adhesion. It was discovered that majority of NIH 3T3 fibroblasts on untreated PTFE were spindle and trigonal shaped. However each of treated surfaces turned out to be rather good substrate for cell spreading and proliferation. For these samples fast cell attachment, growth and well-spreading were registered.

PTFE ion plasma treatment with subsequent α -C:H film deposition seems to be a possible modification to enhance hemo- and biocompatibility of the material.

(134) Effect of Low Oxygen Tension on the Anabolic and Catabolic Activity of Human Articular Chondrocytes from Elderly Individuals

Stroebel S., Barbero A., Wendt D., Candrian C., Lindberg R., Heberer M., Martin I.

Department of Surgery and Research, University Hospital of Basel, Hebelstrasse 20, 4031 Basel, Switzerland

Aging is known to negatively modulate the metabolism of chondrocytes. In this study we investigated whether culturing human articular chondrocytes (HAC) from elderly donors at low oxygen tension during expansion in monolayer or during re-differentiation in pellets would improve their chondrogenic capacity and reduce the expression of specific catabolic mediators.

HAC isolated from cartilage biopsies of four patients (mean age 65 years) were expanded either at 20% or 5% oxygen tension in 10% FBS medium containing TGF β -1/FGF-2/PDGF-BB. Post-expanded cells were subsequently cultured as pellets under both oxygen tensions in medium promoting chondrogenesis. The generated cartilaginous tissues were assessed histologically (Safranin-O), biochemically (glycosaminoglycans [GAG] and DNA), immunohistochemically (collagen-II) and by RT-PCR (collagen-II, aggrecan, MMPs and TIMPs).

HAC expanded under the two oxygen tensions produced tissues with similar poor quality and GAG/DNA contents following differentiation under 20% O₂. Instead, differentiation at 5% O₂ (vs 20% O₂) of HAC expanded at 20% O₂ improved the intensity of staining for GAG and collagen-II, the GAG/DNA content (2.8-fold) and the expression of aggrecan (8.5-fold) and collagen type II (86.6-fold) mRNA. Moreover, pellets cultured under lower oxygen tension expressed lower MMP-1 (7.7-fold) and MMP-13 (3.5-fold) and higher TIMP-1 (3.6-fold) mRNA.

Low oxygen tension applied during re-differentiation in 3D-culture not only enhances matrix production but also reduces the expression of catabolic mediators by HAC, whereas it does not appear to have marked effect if applied during expansion. 3D culture at a more physiological oxygen level might be useful to enhance the outcome of cartilage engineering techniques in aged individuals.

(135) Effect of Rest-Inserted Fluid Flow on Osteoblast Activity in a Collagen-Glycosaminoglycan Scaffold

Plunkett N.A., Jaasma M.J., O'Brien F.J.

Department of Anatomy, Royal College of Surgeons in Ireland, Dublin, Ireland

Trinity Centre for Bioengineering, Trinity College, Dublin, Ireland

Bone cells become accustomed to continuous mechanical loading [1] but their mechanosensitivity can be restored by inserting rest periods into the stimulation pattern [2]. This fact may prove useful in bone tissue engineering in determining the optimum loading regime for stimulating bone cells. This study used a flow perfusion

bioreactor to investigate the effect of combinations of short- and long-term rest periods on osteoblast stimulation in a 3D collagen-glycosaminoglycan scaffold.

Four groups were used: three were cultured under perfusive flow for 25 hours and the fourth was a static control. The three flow groups used 1 hour of 1 mL/min steady flow with short-term rest periods of 0, 10 or 15 seconds inserted after every 10 seconds of flow. All groups used long-term rest periods of 7 hours. Osteopontin (OPN), alkaline phosphatase and cyclooxygenase-2 (COX-2) levels were examined using real time RT-PCR. Cell number was quantified using a DNA assay (Hoechst 33258).

Under steady flow, COX-2 expression increased 17-fold over static levels ($p < 0.05$). OPN expression increased 9-fold for steady flow over 10 second rest-inserted flow ($p < 0.05$). No statistical difference was found between cell number for the groups ($p = 0.137$). Initial results show that combinations of short- and long-term rest periods are not as stimulatory as long-term rest periods. Work is ongoing to examine the effect of longer culture periods on bone formation marker levels.

References

1. Turner, CH (1998). Bone 23(5): 399–407.
2. Batra, NN (2005). J Biomech 38(9): 1909–17.

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(136) Effect of the Pressurizing Process on the Decellularized Aortic Tissue Using Ultra High Hydrostatic Pressurization

Murakoshi A., Kimura T., Funamoto S., Fujisato T., Nakatani T., Kitamura S., Kishida A.

Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Sudugadai, Chiyoda-ku, Tokyo, 101-0062, Japan

Department of Biomedical Engineering, Osaka Institute of Technology, 5-16-1 Omiya, Asahi-ku, Osaka, 535-8585, Japan
National Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka, 565-8565, Japan

The decellularized tissues have been researched as a regenerative bio-scaffold. Generally, they have been prepared through chemical and biological processes using detergents and enzymes. However, some problems, such as ECM denaturation and the toxicity of residual reagents, have reported. So, we have developed a novel physical decellularization method using ultra high pressure (UHP) technology, in which the cells were disrupted by ultra high pressurization and the cellular debris were removed by washing process. The present study aim was to investigate the influence of the pressuring and washing processes on the decellularization and fabric structure of aortic tissue. Porcine aortic tissues were pressurized at different pressurization rate and temperature using a cold isostatic pressurizing machine, and then washed with cell culture medium for several periods. The characteristics of the decellularized aortic tissues were examined by H-E staining, DNA quantification, TEM observation, and mechanical test. The complete decellularization was achieved by the long term of the washing process. Rapid pressurization up to 980 MPa induced the structural disordering of collagen fibrils, in which the wide space of them was observed for H-E staining, whereas in the case of gradual pres-

surization, the collagen fibrils maintained their normal banding pattern, based on TEM observation. Also, the mechanical property of the decellularized aortic tissue was similar to that of native one. From these results, the decellularization method using UHP technology could be useful for preparing tissue-engineered scaffold.

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(137) Effect of Thickness of Nanofiber Scaffolds in Tissue Engineering

Ghasemi-Mobarake L., Morshed M., Nasr-Isfahani M.H., Fesharaki M.A., Karbalayi K., Baharvand H.

Islamic Azad University, Najafabad branch, Isfahan, Iran
Royan Institute, Isfahan campus, Tehran, Iran
Isfahan University of Technology, Textile department, Isfahan, Iran

Collagen is a major natural extracellular matrix component and has a fibrous structure with fiber bundles varying in diameter from 50–500 nm. In order to mimic the structure of native extracellular matrix, nanofiber scaffolds have been an interesting candidate in tissue engineering [1, 2, 3]. The properties of nanofiber scaffolds such as fiber diameter and alignment have some effect on cellular behavior. In this study the effect of thickness of nanofiber scaffolds on the proliferation and morphology of P19 cells was investigated. Nanofiber scaffolds with thickness of 0.1, 0.3 and 0.6 mm were prepared using electrospinning method. Air permeability of scaffolds with various thickness was measured. P19 cells were seeded onto the scaffolds and cultured with DMEM, FCS 10%. SEM, MTT and sectioning of cellular scaffolds were used for investigation of the morphology, cell proliferation and cellular infiltration of P19 cells on the nanofiber scaffolds respectively. Air permeability measurements show that with increasing of thickness air permeability of nanofibers scaffolds decreases. The results of MTT assay, SEM micrographs and sectioning of cellular nanofiber scaffolds show that the proliferation of P19 cells decreases with increasing scaffold thickness. It can be concluded that the increasing of thickness of nanofibers scaffolds can have inhibitory effect on the cell proliferation due to higher packing degree of thicker scaffolds.

References

1. Peter X.M. (2004). Scaffolds for tissue fabrication. Materials today. May: 30–40.
2. Smith L.A. (2004). Nano-fibrous scaffolds for tissue engineering Colloids and Surfaces B:Biointerfaces. 39:125–131.
3. Zuwei M. (2005). Potential of nanofiber matrix as tissue-engineering scaffolds. Tissue engineering. 11: 101–109.

(138) Effect of Three-Dimensional Expansion and Cell Seeding Density on the Cartilage-Forming Capacity of Human Articular Chondrocytes in Type II Collagen Sponges

Francioli S., Candrian C., Martin K., Heberer M., Martin I., Barbero A.

Department of Surgery and of Research, University-Hospital Basel, Hebelstrasse 20, 4031 Basel, Switzerland

We investigated whether the quality of cartilaginous tissues generated *in vitro* by human articular chondrocytes (HAC) on collagen-II sponges can be enhanced by direct expansion of HAC on the biomaterial, as compared to standard monolayer on plastic, or by increasing cell seeding density.

HAC were isolated from the cartilage biopsies of three different patients. Cell constructs were generated by: HAC expansion directly on collagen-II sponges (Chondrocell, Geistlich, CH) for 4 doublings (expansion time: 9 days; density yielded: 35E06 cells/cm³) (group 1); HAC expansion in monolayer for 4 doublings (expansion time: 6 days) and then seeding on the sponges at 35E06 cells/cm³ (group 2); HAC expansion in monolayer for 8 doublings (expansion time: 12 days) and then seeding on the sponges at 35E06 (group 3) or 70E06 cells/cm³ (group 4). Constructs were then cultured for 4 weeks in medium promoting chondrogenesis. The generated tissues were assessed histologically (Safranin-O) and biochemically (glycosaminoglycans [GAG] and DNA).

HAC expanded in collagen-II sponges (group 1) or monolayer (group 2) for 4 doublings generated tissue with fibroblastic appearance and low GAG/DNA. Also HAC expanded in monolayer for 8 doublings generated tissue with poor quality when seeded at 35E06 cells/cm³ (group 3). However, when seeded at 70E06 cells/cm³ (group 4), HAC generated hyaline-like cartilaginous tissues intensely stained for GAG and containing 2.2-fold more GAG/DNA.

High cell seeding density is a crucial factor for chondrogenesis of HAC in collagen-II sponges. Direct expansion on collagen-II sponges did not enhance the quality of the resulting cartilaginous tissues possibly because it did not allow to reach a sufficient cell density.

(139) Effectiveness of Pooled Autoantibody Array in Clinical Diagnostic Autoimmune Diseases by Using Tissue Engineering Technology

Ahangari G.¹, Naderimanesh H.²

¹Department of Molecular Medicine and Immunology, National Institute for Genetic Engineering and Biotechnology, Tehran, Iran

²Department of Proteomics, Faculty of Sciences, Tarbiat Modares University, Tehran, Iran, Ministry of Industries and Mines, High-Tech Industries Center, Tehran, Iran

Introduction: Progress in tissue engineering techniques over the past decade has allowed using living human tissues in clinical treatment. This study has for the first time reported using tissue engineering technology in clinical diagnostic autoimmune test. This investigation was carried on prototypic infertility autoimmune diseases due to autoantibody against spermatozoid cells. Spermatozoa antibodies may be produced systematically and are found in sera of patients. Moreover, antibody bound to spermatozoa in semen should be most relevant to the infertile state. Thus it is important to detect antibody bound to spermatozoa and not rely solely on detection of serum antibody.

Methods: One million donor spermatozoa from 30 healthy individuals were obtained, negative for antisperm antibodies, are incubated with undiluted specimen is then centrifuged, the sperm button washed three times and the spermatozoa resuspend and mixed. Then we added mixture of suspended spermatozoa in fetal calf collagen solution.

Next step after polymerization of collagen we produced three dimensional scaffold included spermatozoa cells. Three dimensional scaffold snap frozen and cut tissues at 4µm cryostat at -20°C. Four slides were used: one for Hematoxyline-eosin and one each for control positive, negative and test. In this investigation, sera of 64 patients were positive for antisperm antibodies and 60 sera of healthy individuals negative for antisperm were used. We add each serum on tissue section in first step and next we add fluorescein labeled antihuman antibody conjugated with fluorescent by using indirect fluorescent antibody method.

Results: Fluorescent emissions occur under microscope if the autoantibody directed against a spermatozoa antigen.

Conclusion: This method provides a new application of tissue engineering to recognize a variety of autoantibodies against different antigen inside and on the cell surface.

(140) Effects of Androgens on Differentiation of Human Muscle-Derived Cells *In Vitro* Are Related to Changes in Local IGF-1 Expression

Solomon A.M., Sinanan A.C.M., Bouloux P.M.G., Lewis M.P.

UCL, Division of Biomaterials and Tissue Engineering, University College London, 256 Gray's Inn Road, London, NW3 2QG, United Kingdom

Introduction: Anabolic androgens have an exciting role in the field of tissue engineering due to their effects on skeletal muscle hypertrophy. The mechanism involved is incompletely understood and may be linked with dynamics of locally acting insulin-like growth factor-1. The present investigations utilised a cell culture model of human skeletal muscle-derived cells to investigate changes in myogenicity following exposure to exogenous androgen +/- IGF-1.

Objectives: To test the hypothesis that androgens increase differentiation of human muscle-derived precursor cells *in vitro* and to assess the effects of androgen +/- IGF-1 on expression of IGF-1 mRNA.

Method: Differentiation of muscle-derived cells was induced using 2% fetal calf serum +/- androgen (testosterone, T, 0-500 nM or dihydrotestosterone, DHT, 0-300 nM) and/or IGF-1 0-50 ng/ml. Immunostaining and RT-PCR were used to assess the effect of androgens and IGF-1 on muscle-specific proteins and IGF-1 mRNA generation after early (3 days) or late (7 days) differentiation *in vitro*.

Results: Significantly increased myogenicity occurred in cells exposed to IGF-1 ($p < 0.02$) in early differentiation. After 7 days' differentiation, supraphysiological doses of T/DHT (+) and IGF-1(+++) were both found to increase fusion index with no observable synergistic effect ($p < 0.05$). Both T and DHT increased IGF-1 mRNA generation ($p < 0.0001$) at the 7 day time point, whereas exogenous IGF-1 ($p < 0.0001$) reduced IGF-1 mRNA concentrations relative to control.

Conclusions: The mechanism whereby androgens induce muscle hypertrophy may be linked to local IGF-1 generation. We have

described a novel system which has the potential for further elucidating the mechanisms by which anabolic steroids act on skeletal muscle.

(141) Effects of Encapsulated Rabbit Mesenchymal Stem Cells on *Ex Vivo* Expansion of Human Umbilical Cord Blood Hematopoietic Stem/Progenitor Cells

Liu Y.¹, Liu T.Q.¹, Fan X.B.¹, Bao C.Y.¹, Ma X.H.¹, Cui Z.F.¹

¹Dalian University of Technology, Dalian R&D Center for Stem Cell and Tissue Engineering, Dalian, 116023, China

²Oxford University, Oxford Centre for Tissue Engineering and Bioprocessing, Department of Engineering Science, Oxford OX1 3PJ, United Kingdom

The expansion of umbilical cord blood mononuclear cells (UCB MNCs) was investigated in a novel coculture system by means of encapsulation of rabbit bone marrow (BM) mesenchymal stem cells (MSCs) in alginate beads (Alg beads). Three kinds of media were applied and the experiments lasted for 7 days. The total nucleated cell density was measured every 24 hours. Flow cytometric assay for CD34+ cells and methylcellulose colony assays were carried out at 0 h, 72 h and 168 h. It was found that the encapsulated MSCs illustrated remarkable effects on UCB MNCs expansion regardless whether serum is present in culture media or not. At the end of 168 h coculture, the total nucleated cell number was multiplied by 15 ± 2.85 times, and CD34+ cells 5.33 ± 0.32 times and colony-forming units in culture (CFU-Cs) 5.6 ± 1.21 times respectively in the serum-free media supplemented with conventional dose of cytokines, which was very similar to the results in the containing 20% serum media. While in the control, i.e. MNC expansion without encapsulated MSCs, however, total nucleated cells density changed mildly, and CD34+ cells and CFU-Cs showed little effective expansion. It is demonstrated that the encapsulated MSCs can support the expansion of UCB MNCs effectively under the experimental condition.

Key words: stem cell expansion; UCB hematopoietic stem/progenitor cells; CD34+ cells *ex vivo* expansion; mesenchymal stem cell; alginate bead

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(142) Effects of Implant Geometry and Surface Modifications on Implant Bone Response

Schouten C., Meijer G.J., Jansen J.A.

Department of Parodontology & Biomaterials, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

Introduction: Bone implant design and surface are important tools to modulate biological responses. Calcium phosphate (CaP) ceramics have been extensively used as carrier materials for osteo-inductive growth factors, such as TGFβ1 [1]. The electrostatic spray deposition (ESD) technique has proven to be a successful method to deposit thin CaP films with controlled morphology on implant materials [2]. In this study two geometrically different titanium implants (screw type and push-in implant) were used. The additional effect of an electrosprayed CaP coating, either or not

enriched with TGFβ1, on the bone formation stimulating capacity of these implants was evaluated.

Materials and Methods: Both implant types were evaluated *in vivo* (i) without modification, (ii) with CaP coating, or (iii) with CaP + TGFβ1, after a twelve week implantation period. Implants were inserted contra-laterally into the trabecular bone at the medial side of the femoral condyle of nine goats. Peri-implant bone mass and bone-implant contact were determined using micro-computed tomography (μCT) and histomorphometry.

Results: mCT evaluation showed a significant increase in peri-implant bone mass for screw type (57%) compared to push-in implants (45%; $p < 0.001$). Additional surface modifications induced no effects on bone response. Quantitative histomorphometrical measurements supported these μCT data.

Conclusion: Implant geometry rather than surface modifications determines the implant bone responses in this goat model.

References

1. Lind et al. Biomaterials. 2001 22(3):189–193.
2. Leeuwenburgh et al. J. Biomed Mater Res A. 2005 74(2): 275–84.

(143) Effects of Pro-inflammatory Stimulation by TNF-α on Osteogenic Differentiation of Adipose Tissue-Derived Stromal Cells

Salamon A.¹, Fiedler T.¹, Adam S.¹, Rychly J.², Peters K.¹

¹University of Rostock, Medical Faculty, Laboratory of Cell Biology, Junior Research Group Bone Regeneration, Germany

²University of Rostock, Medical Faculty, Laboratory of Cell Biology, Germany

Adipose tissue-derived stromal cells (ASC) have multilineage differentiation capacity and can be isolated easily and in large amounts. Therefore, ASC are a promising stem cell source for regenerative medicine. Recent studies revealed a cell fraction within freshly isolated ASC showing distinct expression of CD34 (CD34+ ASC), a well-known marker of hematopoietic stem cells. These cells were able to perform osteogenic and adipogenic differentiation. However, general characteristics of this ASC subpopulation are rather unidentified.

Wound healing is accompanied by an acute pro-inflammatory reaction which is marked by the release of soluble inflammatory factors (e.g. TNF-α). In case of an impaired course of wound healing (e.g. by infection), chronic pro-inflammatory situations can be sustained. Even though chronic inflammatory situations occur frequently, knowledge about effects of long-term pro-inflammatory stimulation on mesenchymal stem cell differentiation is small.

Therefore, we examined the effects of TNF-α on proliferation and osteogenic differentiation of human ASC (both CD34- and CD34+). ASC were treated with TNF-α and osteogenic differentiation inducing medium, containing dexamethasone, ascorbic acid and β-glycerophosphate, for up to four weeks. Cell numbers and osteogenic differentiation markers were quantified.

Treatment of ASC with TNF-α leads to a stronger increase in proliferative activity in both subpopulations than osteogenic stimulation alone. The same is true for osteogenic differentiation. In

general, CD34[−] ASC showed stronger proliferation and osteogenic differentiation than CD34⁺ ASC. Thus, the differentiation degree of ASC subpopulations varied and was additionally influenced by TNF- α .

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(144) Effects of RhGDF-5, RhBMP-2 and a New RhGDF-5 Mutant on Ectopic Bone Formation and Mesenchymal Stem Cell Differentiation

Beyen I., Plöger F., Bormann D., Kramer M., Kasten P., Richter W.

Orthopaedic Surgery, University of Heidelberg, Schlierbacher Landstrasse 220a, Heidelberg, Germany

The use of either growth factors (GF) or bone marrow derived mesenchymal stem cells (MSC) for bone regeneration with beta-tricalciumphosphate (TCP) ceramics was examined in several studies, showing their bone formation capacity *in vivo*. There are few data comparing growth factors concerning their potential of inducing expanded MSC *in vivo*. We investigated this effect using recombinant human growth and differentiation factor-5 (rhGDF-5), a mutant of rhGDF-5 and rhBMP-2 on beta-TCP ceramics for ectopic bone formation in a SCID mouse model.

MSC of 6 donors were expanded and loaded onto beta-TCP scaffolds incorporated with 300 ng/mm³ GF. Scaffolds with GF or MSC alone served as controls. These bioconstructs were implanted subcutaneously into SCID mice (4 scaffolds each) and animals were harvested after 4 and 8 weeks. Osteoinduction was quantified by using Alizarin-Red-S stains, micro-computed tomography (μ CT) scans and specific alkaline phosphatase (ALP) activity.

New bone formation was found in all GF loaded samples with the highest amounts in mutant rhGDF-5 and rhBMP-2. Although wild type rhGDF-5 showed lesser bone formation, cell-loaded scaffolds produced significantly more ALP than empty scaffolds, in contrast to rhGDF-5 mutant and rhBMP-2. *ALU-In Situ* hybridization confirmed the presence of MSC within scaffolds, but new bone due to MSC was found only in the wild type rhGDF-5 and MSC group. In conclusion we can underline the osteoinductivity of GF, especially of mutant rhGDF-5 and rhBMP-2. The wild type rhGDF-5 might be more specific for osteogenic differentiation of MSC *in vivo*.

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(145) Effects of Stem Cells Activation by Cytokines in the Conditions of Toxic Affection of Reproductive System in Mice Males

Zaporozhan V., Kholodkova E., Shcherbatyuk A., Pykhtyeyev D., Perepelyuk N.

Odessa State Medical University, Odessa, Ukraine

Hormonal therapy of the violations of reproductive function can be contra-indicated to the patients with tumors. For medical treatment

of complications at oncologic patients colony-stimulating factors are prescribed at once after ending of course of chemotherapy. But there are no data on the effects of colony-stimulating factors on reproductive system.

The first group of experimental animals was injected with adriablastin (1 mg/kg, twice, intraabdominally), the second in 10 days after introduction of adriablastin were singly entered Gr-CSF in a dose of 100 mg/kg, the third group served as the control.

Introduction of adriablastin causes change of hormonal status so that level of testosterone, progesterone and FSH progressively decrease as compared to the control (32.2%, 22.2% and 61.9% respectively) during the experiment. The level of thyroxin in the blood serum in the beginning rises considerably (32.3%), and by the end of experiment went down on 10% in relation to the control. In the animals of the II group the levels of testosterone and progesterone considerably increase as compared to the control (19.5% and 23.2% respectively). At the same time there was the moderate increase of the FSH level (40.5%) as compared to the control. The level of thyroxin rose on 21.3% in comparison to the control.

Introduction to the animals Gr-CSF the processes of renewal of normal level of hormones were considerably accelerated, that shows protective action of colony-stimulating factor on reproductive system. So, correction of violations of function of reproductive organs with the cytokines is possible.

(146) Effects of VEGF Loaded Hyaluronic Acid Based Hydrogels on Ischemic Heart Regeneration

Park Y., Yoon S.J., Fang Y.H., Lim C.H., Kim B.S., Sun K.

Department of Biomedical Engineering, Korea Artificial Organ Center, College of Medicine, Anam 5 Ga, Sungbuk Gu, Seoul, 136-705, Korea

Myocardial infarction is one of the important causes of heart failure. Stem cells and growth factors have been used for improving heart function. Biodegradable scaffolds have been used for efficient delivery of cells and growth factors. We used injectable hyaluronic acid based hydrogel to deliver VEGF for regeneration of myocardial infarction. Rat left anterior descending (LAD) coronary artery was ligated for the infarction. Four groups were set for the experiments; sham operation (SO), infarction (MI), hydrogel injected, and VEGF loaded hydrogel group ($n = 6$ in each group). After two weeks, hyaluronic acid based hydrogels loaded with 1 microgram of vascular endothelial growth factor (VEGF) were injected to the epicardium of infarcted area. Heart function was analyzed by measuring pressure and flow. Compared to sham operation group, gel injected group showed the improved ejection fraction and cardiac output. VEGF-loaded gel group also showed the improved heart function. VEGF loaded group showed slight improvement of heart function compared to gel injected group. However, there was no significant difference between two groups. Histological analysis shows that wall thickness is recovered to the normal level. Capillary formation in the border area increased significantly in gel injected and VEGF-loaded gel groups. Gel injected and VEGF loaded gel group showed the significant increase compared to the MI. Hyaluronic acid based hydrogel alone improved the heart function and regenerate the myocardium and can be used for delivering vehicles for VEGF.

(147) Electric Fields Guide Neuron Migration, Polarization and Neurite Orientation

Yao L.^{1,2}, McCaig C.¹, Zhao M.¹

¹School of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom

²National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland

Introduction: Effective and directed neuron migration is critical for development and repair in the central nervous system. Endogenous electric fields (EFs) are widespread in developing and regenerating tissues. Application of EFs directs cell migration of many types of cells and orient neurite growth. However the EFs directed neuronal cell body migration and the establishment of cell polarity and neurite re-orientation in migration neurons have not been demonstrated.

Materials and methods: Dissociated rat hippocampal neurons were cultured on poly-L-lysine and laminin coated dishes. The neurons were exposed to small applied electric fields and the migration was recorded with a time-lapse imaging system.

Results: 1. Rat hippocampal neurons migrate toward cathode pole in an applied EF. The leading growth cone-like process, Golgi apparatus and centrosome re-distribute asymmetrically to face the cathode. The cathodal polarization of neurons occurred in parallel with cathodal neuronal migration. 2. Inhibition of ROCK (Rho-associated protein kinase) and PI3 kinase (phosphoinositide-3 kinase) decreased the directedness and speed of guided neuronal migration in EFs. It also decreased leading growth cone orientation and Golgi polarization in the neurons in response to EF.

Conclusion: This work therefore demonstrates the establishment of cathodal polarization of both neuronal cell morphology and intracellular structures in an applied EF and EF directed hippocampal neuron polarization and migration are signaled by PI3k and ROCK pathways. This raises the possibilities that electric fields may be used as a potential cue to direct neuronal migration in repair of the central nervous system.

(148) Electrical Stimulation of Muscle Progenitor Cells to Optimize Differentiation and 3D Tissue Culture

Langelaan M.L.P., Boonen K.J.M., Post M.J., Bouten C.V.C., Baaijens F.T.P.

Eindhoven University of Technology, Department of Biomedical Engineering, Eindhoven, The Netherlands

Skeletal muscle is a complex tissue that consists of different cell types, nerves and vessels to perform its biomechanical function. So far, the culture of muscle tissue mainly starts with muscle progenitor cells, since these are the cells involved in skeletal muscle regeneration *in vivo*. External stimuli are required to induce determination and differentiation of the progenitor cells into skeletal muscle cells. Traditionally, researchers have applied biochemical stimuli by altering the chemical constitution of the culturing medium. However,

biophysical stimuli, such as electrical stimulation, also play an important role in the maturation of skeletal muscle cells.

The effects of electrical stimulation were systematically investigated using a commercially available multi-channel field-stimulator (C-Pace, IonOptix Corporation) on 2D cultures of C2C12 murine skeletal myoblasts. When appropriately stimulated, these cells fuse to multinucleated myotubes. Electrical stimulation of the cultures for 48 hours with bipolar stimuli of 10 V, 6 ms and 2 Hz, revealed contracting myotubes. Compared to non-stimulated controls, the electrically stimulated myotubes appeared more mature, based on morphology and the production of the transcription factor myogenin.

Apart from optimizing the electrical stimulation protocols, future experiments will include other biophysical stimuli, such as substrate properties, to investigate their relative contributions on muscle differentiation, both in 2D and 3D environments.

(149) Electrophysiological Responses of HMSCs and Bone Cells to Magnetic Particle Tagging

Kirkham G.R., Elliot K.J., Knight M., Keramane A., Akhtar B., Salter D.M., Dobson J., El Haj A.J., Cartmell S.H.

Institute for science and technology in medicine, Keele University, University Hospital of North Staffordshire, Thornburrow Drive, Stoke-on-Trent, Staffordshire, ST4 7QB, United Kingdom

Magnetic particle tagging has been proposed as a method for controlling cell behaviour using remote magnetic fields, and has considerable potential for tissue engineering applications that involve mechanical conditioning regimes. Magnetic microparticles (coated with Arg-Gly-Asp) were attached to the membranes of MG63 and human mesenchymal stem cells. An oscillating magnetic field was then applied, allowing force application at controlled frequencies. The electrophysiological responses of each cell type were monitored before and after force application. A series of ion channel antagonists were then employed in order to investigate the early mechanotransduction pathways involved. Finally, the degree of particle displacement was tracked using confocal microscopy.

The application of force elicited hyperpolarisation responses in both cell type (a 65% and 66% increase in hyperpolarisation respectively). Samples exposed to force in the presence of Gadolinium showed no changes in membrane potential, indicating a key role of stretch activated channels. Large conductance calcium-activated potassium (BK) channels were also shown to mediate the response, as samples containing Tetraethylammonium chloride and 4-Aminopyridine did not demonstrate significant hyperpolarisation responses.

The use of Nifedepine indicated that extracellular calcium may not play a key role in mediating the hyperpolarisation. Conversely, blocking intracellular calcium release from the endoplasmic reticulum, with Thapsigargin, did significantly eliminate the hyperpolarisation response. Finally, the degree of magnetic particle displacement was quantified using a digital image correlation analysis. This demonstrated a significant degree of particle movement on the cell membrane, in the order of 0.6–0.7 μm , after the application of the magnetic field.

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(150) Electrospun Scaffold for Blood Vessel Tissue Engineering

Liu J., Lee S.J., Yoo J.J., Soker S.

Wake Forest Institute for Regenerative Medicine, Wake Forest University Health Sciences, United States of America

There is significant potential for using electrospinning technology to fabricate scaffolds for blood vessel tissue engineering applications. Specifically, the method is relatively simple, highly controllable and reproducible, and further, can create a continuous, intertwined fibrous structure. In this study, we further explored the utility of electrospun scaffolds for vessel tissue engineering.

Vascular scaffolds were fabricated by electrospinning with polymer blends of collagen type I from calf skin and poly-ε-caprolactone (PCL, Inherence viscosity = 1.77 dL/g) with the ratio of 50:50 in weight. The solutes were mixed in 1,1,1,3,3,3-hexafluoro-2-propanol (99+%) (HFIP) at a total solution concentration of 5% (w/v). Scaffolds were crosslinked in the vapor of glutaraldehyde solution overnight to increase stability and strength. The electrospun PCL/Collagen fibers showed diameters of (768 ± 48) nm and a random orientation of fibers. Collagen type I demonstrated a uniform distribution. The scaffold had a high tensile strength (3.72 MPa) and good elasticity, with a burst pressure of 4860 mmHg, or roughly twice that of native blood vessels. Viability assays revealed an average of 88% of bovine endothelial cells survived on the scaffold. Scanning electron microscopy and histology confirmed a confluent layer of endothelial cells on the inside and smooth muscle cells on the outside of the scaffold after 3 days.

This study demonstrates that the electrospun scaffolds are biocompatible and possess appropriate mechanical integrity and structural characteristics. The PCL/Collagen scaffold provides a favorable environment for supporting the growth of vascular wall cells. Collectively, this study demonstrates the feasibility of electrospinning as an effective fabrication technique for cardiovascular grafts.

(151) Embryonic Stem Cell Transplantation in Multiple Sclerosis

Smikodub O.I.

Cell Therapy Clinic of the National Medical University of Ukraine and Embryonic Tissues Center EmCell, Solomenskaya Str. 17, POB 80, Kiev 03110, Ukraine

Under observation were 24 patients with relapsing-remitting MS, duration of the disease -2, 13 ± 0, 54 years, duration of remissions -3, 4 ± 1, 2 months. Most often, observed were pyramidal and sensory disturbances, ophthalmological symptoms.

For treatment, used were embryonic stem cell suspensions containing stem cells of mesenchymal and ectodermal origin obtained from growth zones of 4–8-week-old embryonic cadavers' organs. Suspensions were administered in the amount of 1–3 ml, cell count 0, 1–100 × 10⁵/ml. After the treatment, 70% of patients reported the Syndrome of Early Post-Transplant Improvements: decreased weakness, improved appetite and mood, decreased depression. For several months after the treatment, positive changes were reported in the following aspects: nystagmus, convergence disturbances, spasticity, and coordination. Improvements of dys-

arthria, dysphagia and ataxia, took more time. In general, the treatment resulted in the improved range and quality of voluntary motions, normalized muscle tone, decreased fatigue, and improved quality of life. Besides, 87% of patients reported no relapses, no aggravation of neurological symptoms, and no further progression of the disease. MRI performed 1–2 years after the treatment, revealed considerable diminution of focal lesions, mean by 31%, diminution of gadolinium enhanced lesions by 48%; T2-weighted images showed marked decrease of the foci's relative density.

(152) Embryonic Stem Cells in Insulin Resistance Decrease in Patients with Impaired Glucose

Smikodub O.I., Novytska A.V.

Cell Therapy Clinic of the National Medical University of Ukraine and Embryonic Tissues Center EmCell, Solomenskaya Str. 17, POB 80, Kiev 03110, Ukraine

For correction of carbohydrate and lipid metabolism, 11 patients (6 men and 5 women, age 52.7 ± 4.6 years) with insulin resistance syndrome: IGT (fasting glycemia (FG)—5.8 ± 0.2 mmol/l, in 2-hr of OGTT—9.8 ± 1.1), stage I-II hypertension, BMI—28.4 ± 2.3 kg/m², ratio waist/hips—0.98 ± 0.05 (men), 0.91 ± 0.06 (women) and dyslipidemia—elevated cholesterol (5.96 ± 0.74 mmol/l), triglycerides (1.89 ± 0.43), increased LDLP (3.74 ± 0.56) and decreased HDLP (1.49 ± 0.23) were treated by transplantation of embryonic stem cells. Insulin resistance was estimated by HOMA-IR, 3.85 ± 0.54. Control group consisted of 8 healthy people aged 50.3 ± 2.4 with NGT (FG: 5.1 ± 0.4 mmol/l, in 2-hr of OGTT: 6.8 ± 0.3); HOMA-IR—1.97 ± 0.19.

Intravenously transplanted were embryonic hematopoietic/non-hematopoietic mesenchymal and endodermal stem cells obtained from growth zones of 4–8-week-old cadaverous embryos' internal organs (amount: 1.0–3.0 ml, cell count: 0.1–100 × 10⁵/ml).

Observation period was 1–3 years. During the first month 91% of patients reported decreased weakness and improved workability; in 2–3 months, gradual decrease of OGTT indices. Evident improvement was noted in 7–9 months: normalization of FG—5.2 ± 0.6 mmol/l, in 2-hr of OGTT—7.4 ± 0.4 mmol/l, moderate decrease of fasting insulinemia by 29.3 ± 0.6%, marked decrease of stimulated insulinemia—in 30 minutes of OGTT—by 55.4 ± 1.7%, in 2-hr—by 71.1 ± 2.6%, and decrease of HOMA-IR by 40.3 ± 0.8% (2.33 ± 0.16). Normalization of blood lipids was reported in 73% of patients after 9–12 months (decrease of triglycerides, cholesterol, LDL and HDL increase).

(153) Embryonic Stem Cells in Metabolic Syndrome

Smikodub O.I., Novytska A.V.

Cell Therapy Clinic of the National Medical University of Ukraine and Embryonic Tissues Center EmCell, Solomenskaya Str. 17, POB 80, Kiev 03110, Ukraine

Observed were 12 patients, 7 men and 5 women (mean age: 58 ± 6.3), with clinical manifestations of metabolic syndrome. All patients presented stage I-II hypertension, carbohydrate metabolism disturbances: impaired glucose tolerance (IGT)—8 and mild type II diabetes mellitus (DM) (morning fasting hyperglycemia,

aglycosuria)–3, with hyperinsulinemia and elevated C-Peptide (4.7 ± 1.6 ng/ml). Lipid metabolism disturbances were reported in all the patients: elevated cholesterol, triglycerides, increased concentration of low density lipoproteids (LDL), very low density lipoproteids (VLDL), decreased concentration of high density lipoproteids (HDL), and abdominal obesity (BMI: 30.8 ± 1.6 kg/m²), waist (relatively W: 94.8 ± 2.3 , M: 105.7 ± 2.9 cm).

Transplanted were embryonic hematopoietic and non-hematopoietic mesenchymal and endodermal stem cells obtained from germ layers of 4–8-week-old cadaverous embryos' internal organs and sorted thereafter. 1–3 ml of cell suspensions were administered intravenously, cell concentration: $0.1\text{--}100 \times 10^5$ /ml.

Patients were observed for 1–5 years. In 1–2 months, 83% of patients reported gradual BP decrease with subsequent stabilization in the course of the next 2–3 months: 135–140/80–90 mm Hg, with parallel 1.5–2-fold decrease of hypertension medications. In 7–9 months, glucose tolerance test (glycemia level 2 hours after glucose load was 7.2 ± 0.3 mmol/l) revealed the improvement of carbohydrate metabolism in IGT patients. Decrease of morning hyperglycemia in type II DM patients usually started in 2–3 months, normalization was observed in 5–7 months. C-Peptide concentration gradually decreased, beginning from after 2–3 months, and normalized within 6–9 months. Normalization of blood lipids was reported in 75% of patient after 9–12 months, with parallel decrease of triglycerides, cholesterol, LDL, VLDL, and HDL increase.

(154) Embryonic Stem Cells in Pernicious Decompensated Type 2 Diabetes Mellitus

Smikodub O.I., Novytska A.V.

Cell Therapy Clinic of the National Medical University of Ukraine and Embryonic Tissues Center EmCell, Solomenskaya Str. 17, POB 80, Kiev 03110, Ukraine

Group of 11 DM patients (4 women and 7 men) with beta cell deficiency and such complications as micro- and macroangiopathy, diabetic ketoacidosis caused by maximal dosages of glycemic medications, and in need for insulin therapy, mean age being 54.3 ± 8.2 years, duration of the diagnosed DM— 18.7 ± 5.3 years. Patients were treated with maximal dosages of sulphonylureas medications in combination with other glycemic medications. In all patients, mean C-Peptide was reported to be 1.2 ± 0.7 ng/ml, HbA1C concentration— $9.7 \pm 2.2\%$.

Patients were treated by transplantations of hematopoietic and non-hematopoietic mesenchymal and endodermal embryonic stem cells sorted out from growth zones of 4–8-week-old cadaverous embryos' internal organs. Suspensions were administered intravenously, in the amount of 0.5–3.0 ml, cell count being $0.1\text{--}100 \times 10^5$ /ml.

82% of reported normal urine status and considerably improved general state. In the course of 2–3 months after treatment, noted were decreased glycosuria and daily glycemia; it was possible to decrease maximal dosage of glycemic medications. Lasting compensation of carbohydrate metabolism was achieved in 8–12 months after the treatment. By this time, C-Peptide increased 1.8–2.2-fold in 64% of patients, while HbA1C concentration decreased by 26–30%. Decreased were also manifestations of diabetic retinopathy, nephropathy, and polyneuropathy. In 3–4 months after the initial treatment, 27% with hypertension reported lasting decrease of blood pressure.

Subsequent transplantations were performed in 60% of patients in 12–16 months, with the aim of DM compensation and stabilization of the results of the initial course of treatment.

(155) Embryonic Stem Cells in Rheumatoid Arthritis

Smikodub O.I., Demchuk M.P.

Cell Therapy Clinic of the National Medical University of Ukraine and Embryonic Tissues Center EmCell, Solomenskaya Str. 17, POB 80, Kiev 03110, Ukraine

For 9 years, followed-up were patients with 1.5–20-year history of confirmed rheumatoid arthritis (RA) with drug intolerance or inefficiency of routine methods, and marked limitation of functional capacity. For treatment, used were cryopreserved embryonic stem cell (ESC) suspensions containing hematopoietic and non-hematopoietic mesenchymal stem cells prepared from organs of 4–8-week-old embryonic cadavers. Total nucleated cell count— $10\text{--}400 \times 10^6$ /ml, mononuclear cell count— $10\text{--}100 \times 10^4$ /ml. Upon ESC administration, all patients reported the Syndrome of Early Post-Transplantation Improvements manifested by decreased general weakness, subsidence of pain, improved and more optimistic mood, improved appetite and normalized sleep formula. This method of treatment allowed for subsidence of inflammatory activity from high (14 points) to minimal (3 points), and clinical remission in 85% of cases.

Over a period of one year, patients under observation reported decrease of pain, articular, and inflammatory indices—main clinical criteria of RA manifestations, functional capacity increase manifested by the ability of performing everyday activities patients were unable to perform prior to this treatment, improved gait, ability to use public transport and continue full-time work. Subsequent transplantations were performed in 62% of cases: 80% of them were aimed at functional capacity and life quality improvement, and only 20% were performed for the decrease of the disease activity.

Immunocorrecting effect of ESC was observed in all the patients. Reported was increase of T-suppressor count (CD8+); helper/suppressor ratio CD4+/CD8+ decreases over a period of 360 days. Prior to the treatment, rheumatoid factor titer amounted to 3.2 U, and within 360 days, it decreased to 1.02 U.

(156) How to Spy on Cells in Contact with Biomaterials Which Serve as Scaffolds? Tricky Method for Cell Visualisation and a Control System for Quantitative Analysis in 3D Systems

Lewandowska-Szumiel M.¹, Woźniak P.¹, Chróścicka A.¹, Jaegerman Z.²

¹Department of Biophysics and Human Physiology, Medical University of Warsaw, Poland

²Institute of Glass and Ceramics, Warsaw, Poland

Since culture systems evaluated for the purpose of tissue engineering differ from standard culture, there are several problems with reliable evaluation of cell behaviour in direct contact with biomaterials in such systems. Observation of cell morphology may be difficult. Besides, quantitative analysis of cell behaviour in 3D systems *in vitro* is problematical due to the lack of a good reference. Simple, tricky methods of cell visualisation as well as a

positive control for tests performed in culture on 3D scaffolds are being proposed.

The first is based on a possibility of cells visualisation due to the aldehyde-related fluorescence, in a combination with the cells localisation in SEM both, in SE mode and by the X-ray characteristic signal-based mapping. It can be achieved due to the arsenic presence in cells fixed in 2,5% glutaraldehyde in sodium cacodylate. A suitable system of a positive control in a prolonged culture of cells in experimental 3D systems is based on a perfect tolerance of cells towards 3D corundum scaffolds.

Experimental examples of the efficacy of proposed resolutions in culture systems on various scaffolds are being presented.

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(157) Endoblin Expressed by Human Bone Marrow Mesenchymal Stem Cells Post *Ex Vivo* Replication Defective Adenovirus BMP-2 Mediated Gene Transfer

Chang S.C.N., Lin T.M., Chung H.Y., Jeng L.B.

Department of Plastic Surgery, China Medical University Hospital, Taichung, Taiwan

Autologous vascularized bone grafts, allografts, and biocompatible artificial bone substitutes each have their shortcomings. The bones regenerated using peptides recombinant human bone morphogenetic proteins (rhBMPs), demineralized bone powder or combinations of both are in general, small in size and do not meet the need. Current trend is to generate bones in desired size and shape utilizing bone marrow mesenchymal stem cells (MSCs) by tissue engineering procedures. Tissue engineering integrating *ex vivo* gene therapy and polymer science may have clinical significance to bone regeneration application. This study aims to understand *in vitro* cellular differentiation post adenoviral infection.

The experimental group was composed of *ex vivo* replication defective adenovirus BMP-2 mediated gene transfer to the expanded human bone marrow mesenchymal stem cells (MSCs). The controls were performed using human MSCs. We tested the parameters in flow cytometry as CD166, CD105 and CD34 post infection 2, 3, 6 and 9 days.

Comparison between the experimental and control groups, there were no difference in the expression of CD34. CD166 showed no significant increase in the experimental group. Both groups demonstrated less decrement of CD105 in the experimental group.

This data evidenced that *ex vivo* replication defective Adenovirus mediated human BMP-2 gene transfer to MSCs enhances the expression of CD105 (endoglin) which is an accessory protein of multiple kinase receptor complexes of the TGF- β superfamily. Further study in MSC differentiation will be conducted for further feasible regional cell therapy by *ex vivo* replication defective adenovirus BMP-2 mediated gene transfer.

(158) Engineered Muscle Using the C2C12 Cell Line as a Tool for Studying Muscle Physiology and Function

Khodabkhus A., Baar K.

University of Dundee, JBC, Dow Street, Dundee DD1 5EH, United Kingdom

Clinical application of engineered skeletal muscle is decades away due to limitations such as the lack of vascularisation and innervation. The aim of this study was to optimise conditions for engineering muscle from the C2C12 cell line using fibrin gel casting. Due to rapid breakdown of pure fibrin gels by transformed cells, the number of cells, the media, and the composition of the gel were optimised for maximal force production over time. Constructs engineered with aprotinin, an inhibitor of the fibrinolytic enzyme plasmin, in the gel produced peak twitch force (Pt) $12.6 \pm 1.8 \mu\text{N}$ and peak tetanic tension (Po) $20.1 \pm 2.1 \mu\text{N}$ after 7 days with force decreasing over time prior to construct failure at 18.4 ± 3.2 days. The addition of genipin, a cross-linker, to the gel gave Pt = $11.5 \pm 1.9 \mu\text{N}$ and Po = $35.1 \pm 10.9 \mu\text{N}$ after 7 days and maintained force production over time so that at 3 wks constructs generated Pt = $17.8 \pm 4.8 \mu\text{N}$ and Po = $45.6 \pm 10.9 \mu\text{N}$. In conclusion we have engineered skeletal muscle from the C2C12 cell line which fully form in 10 days, survive in culture for 3 wks and can be used as a tool to study the effects of genetic alterations, pharmacological interventions, vascularisation and innervation on muscle physiology and function.

(159) Engineering and Testing 3D Stiffness Gradients in Collagen Scaffolds Towards Durotactic Control

Hadjipanayi E., Mudera V., Brown R.A.

Institute of Orthopaedics and Musculoskeletal Science, Royal National Orthopaedic Hospital, Brockley Hill, Stanmore HA7 4LP, United Kingdom

Matrix stiffness has been implicated in cellular migration, proliferation and adhesion. This study aimed to engineer and test a continuous 3D stiffness gradient which can be used as a model to study the importance of differences in matrix stiffness on such processes. Collagen scaffolds with a gradient of matrix stiffness were prepared by casting 7 ml of collagen solution in wells which were inclined at 45° . After setting, the wedge-shaped gels were placed horizontally and compressed vertically to produce sheets of a desired (0.25 mm) uniform thickness, but with increasing density along the length of the sheet. Dynamic mechanical analysis was carried out on 1 mm wide strips ($n = 3$) obtained from the two ends and the middle of each sheet, to measure changes in elastic modulus, and accumulation of sepharose marker beads was measured to determine the density gradient formed. The elastic moduli, 2305 ± 693 and $1057 \pm 487 \text{ kPa}$ at the stiff and soft end respectively and $1835 \pm 31 \text{ kPa}$ in the middle, represented a near linear reduction in modulus along the sheet. Mean sepharose bead density along the same gradient fell from 71 ± 12 to 10 ± 1 at the stiff and soft end respectively and was 19 ± 5 in the middle. This indicates the nature of the density gradient in the collagen construct, corresponding to the stiffness gradient. The ability to engineer a continuous 3D stiffness gradient together with precise control of both its absolute and relative properties provides an effective model for studying cellular behaviour, such as nerve or endothelial guidance, *in vitro* and designing mechanically stable biomimetic structures for use in engineering fine tissue structures.

(160) Engineering Cartilaginous Explants Using Human Stro-1+ Progenitors, Alginate Scaffolds and Perfusion Bioreactors

Tare R.S., Forsey R.W., Chaudhuri J.B., Oreffo R.O.C.

University of Southampton, Bone and Joint Research Group, Southampton General Hospital, Southampton, SO16 6YD, United Kingdom

University of Bath, Centre for Regenerative Medicine, Department of Chemical Engineering, Bath, BA2 7AY, United Kingdom

The limited repair capability of articular cartilage is a significant challenge in the treatment of cartilage injury and degeneration. Tissue engineering therefore may provide an alternative solution for cartilage reconstruction and replacement. We examined the potential of Stro-1+ progenitor cells immunoselected from human bone marrow, in comparison to human articular chondrocytes and the murine chondrogenic ATDC5 cell line, to generate 3D cartilage constructs. Cells were encapsulated in alginate/chitosan beads and cultured for 28 days under static conditions or within a perfused bioreactor operating at a flowrate of 1 ml/day.

Alcian blue-stained sections of 28-day cartilaginous explants from the three cell types cultured under static and bioreactor conditions were histologically reminiscent of hyaline cartilage. Staining with Cell tracker green and Ethidium homodimer confirmed that the majority of cells within explants were viable and metabolically active. Real-time media analysis for monitoring changes in cell metabolites supported this finding and highlighted significant differences between alginate samples and cell culture controls. Expression of SOX-9 and Type II collagen proteins by the explant cells was analysed by Western blotting. Robust expression of SOX-9 and Type II collagen was observed in ATDC5 explants cultured in static and bioreactor conditions. Although SOX-9 expression was induced in explants of human articular chondrocytes and Stro-1+ cells under both culture conditions, expression of Type II collagen was observed only in explants cultured in perfused bioreactors.

The study has highlighted importance of 3D environments in conjunction with optimal perfusion flowrates to stimulate chondrogenic differentiation in human mesenchymal progenitor populations.

(161) Engineering Functional Collagen Scaffolds by Mechanical Loading

Cheema U., Chuo C.B., Sarathchandra P., Nazhat S.N., Brown R.A.

UCL, Tissue Repair and Engineering Centre, Institute of Orthopaedics, Stanmore Campus, Brockley Hill, HA7 4LP, United Kingdom

The material properties of connective tissues fulfil a variety of tensile, compressive, shear and torsional load bearing functions. These are achieved by standard adaptations of the fibrous, anisotropic form of their collagen component. A key determinant of that is the distribution of fibril diameters, which vary from tissue to tissue *in vivo*. We have tested the idea that fibril diameter can be regulated directly, using mechanical loading to promote fibril fusion in plastically compressed collagen materials.

Acellular collagen gels were routinely compacted by a combination of compression and blotting. Cyclical load was applied parallel to the tethered axis of the collagen gels. Gels were ana-

lysed for fibril diameter (electron microscopy) or quasi-static tensile mechanical properties directly after treatment.

The median collagen fibril diameter increased with increasing load cycle number. The median baseline fibril diameter (1 cycle) was 29 ± 4.6 nm and this increased >2 fold to 70 ± 10 nm after 144 cycles ($p < 0.001$). Break stress and modulus increased by 4.5 and 2 fold respectively.

This study represents the first demonstration, to our knowledge, that both fibril diameter and overall material properties can be directly controlled, without cells, through mechanical loading. This would suggest that material properties of the natural collagen polymers *in vivo* may also be controlled by a combination of local, cell generated strains and external loading. It also redirects how we can engineer biomimetic collagen materials for implants by providing previously impossible cell-independent control of mechanical properties.

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(162) Engineering Microchannels into Collagen Constructs with Soluble Phosphate Glass Fibres for Neural Tissue Repair

Alekseeva T.¹, Allovskaia A.¹, Abou Neel E.A.², King V.³, Knowles J.C.², Brown R.A.¹

¹University College London, Tissue Repair and Engineering Centre, Institute of Orthopaedics, London HA7 4LP, United Kingdom

²UCL, Eastman Dental Institute, Division of Biomaterials and Tissue Engineering, 256 Gray's Inn Road, London, WC1X 8LD, United Kingdom

³Queen Mary University of London, Neuroscience Centre, Institute of Cell and Molecular Science, London, E1 2AT, United Kingdom

We report here a novel implantable device for neural tissue engineering based on plastic compressed (PC) collagen. PC collagen is a novel technique for the rapid fabrication of dense collagen biomimetic tissues (1). Dissolution of phosphate glass (PG) fibres compressed into collagen gels produce microchannels (2) but products from fast dissolving glasses may be detrimental to seeded neural cells. In this study we tested the viability of Schwann cells (SC) in this system.

SC-seeded PG-collagen constructs were prepared as previously described (2) (PG fibre diameter 30–40 μ m, composition ratio: 0.5 (P₂O₅) : 0.25 (CaO) : (Na₂O); distilled water dissolution time 8–10 hrs). PG-constructs were fabricated with 1×10^6 SC, cultured for 1–3 days and stained with both ethidium homodimer-1 and for p75 (SC marker antigen) to determine live-dead cell ratio and SC proportion (with confocal microscopy).

Percentage of dead cells in constructs with PG fibres, at 36% was statistically greater than in constructs with no PG fibres (16%) after 3 days *in vitro* ($p < 0.005$: i.e. 20% additional SC death). However this left 64% SC viability in the presence of highest levels of PG dissolution products suggesting that more slowly dissolving PG needed here (>1 week) will generate channels but have little cell toxicity.

References

1. Brown R.A. et al. (2005) Ultrarapid Engineering of Biomimetic Materials and Tissues: Fabrication of Nano- and Microstructures by Plastic Compression, *Adv. Funct. Mater.* 15(11). 1762–1770.

2. Nazhat S.N. et al. (2007) Controlled Microchannelling in Dense Collagen Scaffolds by Soluble Phosphate Glass Fibres, Biomacromolecules. 8(2). 543–551.

Acknowledgements: Work was funded by BBSRC and EPSRC.

(163) Engineering of Hypertrophic Cartilage with and without Poly(glycolic Acid) Scaffolds

Kwarciak A., Crawford A., Brook I.M., Hatton P.V.

Centre for Biomaterials & Tissue Engineering, University of Sheffield, School of Clinical Dentistry, Claremont Crescent, S10 2TA, Sheffield, United Kingdom

Introduction: Engineered hypertrophic cartilage template that can mineralize *in vitro* or *in vivo* represents an alternative approach for bone tissue engineering. Hypertrophic cartilage can survive in a relatively hypoxic environment, which may allow more time for vascularization and mineralization of the engineered graft to develop post-implantation. The aim of this research was to utilize different cell culture conditions to investigate whether they promote generation of a cartilage construct with characteristics of hypertrophic tissue.

Methods: Chondrocytes were isolated from rat sternal or nasal cartilage, their numbers expanded and then cultured as pellets or on PGA scaffolds under conditions intended to promote chondrogenic differentiation. Chondrocyte differentiation was evaluated by immunolocalisation of collagens (types I, II and X) and histochemical detection of proteoglycans, calcium deposition and alkaline phosphatase.

Results: Successful chondrogenic re-differentiation was observed in pellets and on PGA scaffolds of both cell types. However, alkaline phosphatase activity and collagen X expression were detected only in constructs engineered using a PGA scaffold.

Conclusion: Only chondrocytes seeded onto PGA scaffolds expressed characteristics of hypertrophic cartilage. This suggested that a cell-on-scaffold approach may be more useful than scaffold-free cell pellets for the generation of hypertrophic cartilage with therapeutic potential. Ongoing work is directed at determining culture conditions to accelerate hypertrophic differentiation in nasal chondrocytes.

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(164) Engineering Vascularized Microtissues for Bone Regeneration

Rivron N.C., Rouwkema J., Bettahalli Narasimha M.S., Stamatialis D., Wessling M., De Boer J., Van Blitterswijk C.A.

Department of Tissue Regeneration, Twente University, The Netherlands

The formation of dense functional tissue allowing rapid vascularization is critical in most cell based tissue engineering applications. Most strategies rely, today, on the ingrowths of blood vessels from the host (angiogenesis) which is possibly too slow for optimal implant survival and integration. A potential approach to circumvent this problem is to combine the implant with a prevascular network *in vitro* that can rapidly anastomose to the host blood system after implantation [1].

In this study, we hypothesize that spherical microtissues of cocultured endothelial cells (HUVEC or patient's own EPC) and osteoprogenitor cells (BmMSC) grown in a hanging drop can result in the formation of prevascularized microtissues *in vitro*. We show, in a multiple layer model, a complex morphogenetic process leading to the formation of an homogenous precapillary network (vasculogenesis) [2]. We further demonstrate that a fraction of the MSC are involved in building the precapillary network and driven towards the endothelial lineage (CD31+, vWF+). This adds a substantial clue to the versatility of MSC and their potential to be used as a source of endothelial cells.

This study depicts the possibility of building, *in vitro*, dense and functionalized units designed for improved *in vivo* bone regeneration. By adding clues to the endothelial potential of MSC, it also raises the possibility to engineer vascularized bone tissue from a single cell source.

References

1. S. Levenberg and Al. (2005), Nature Biotechnology, 23, pp. 879–884.
2. J. Rouwkema and Al. (2006), Tissue Engineering, 12, pp. 2685–2693.

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(165) Enhancement of Cell Migration in Culture of Human Epithelial Cells on Surface with EGF and D-glucose Display

Kim M.-H., Kino-oka M., Kawase M., Yagi K., Taya M.

Osaka Univ, Dept Biotechnology, Osaka 5650871, Japan
Osaka Univ, Div Chemical Engineering, Osaka 5658531, Japan
Osaka-ohsaka Univ, Faculty of Pharmaceutical Sciences, Osaka 5848540, Japan
Osaka Univ, Grad School Pharmaceutical Sciences, Osaka 5650871, Japan

The D-glucose displayed surface can offer a promising design of stimulators for many anchorage-dependent cells by means of surface-localized array of ligands targeting receptors on cell membranes. In this study, we investigate the influence of EGF and D-glucose display on the morphology and migration of human epithelial cells to clarify their synergistic effect.

By the time-lapse observation the cells on EGF/D-glucose-displayed surface caused active migration, accompanied with periodic changes in the morphologies of round- and stretch-shapes. However, on the surface with displaying EGF and L-glucose, the cells were found to be less motile with exhibiting round-shaped morphology.

To confirm the activation of EGF receptor associated with cellular bindings mediated by integrin and GLUT, fluorescent staining of phosphotyrosine PY20 and vinculin was conducted. On the

surface with displaying EGF and D-glucose, the cell exhibited increment of the phosphorylated tyrosine expression and formation of focal contacts with vinculin spots. This results support the consideration that the D-glucose display on surface permits the cells to be in close contact with the surface via grasping of GLUTs on plasma membrane, being attributable to the up-regulation of EGF receptor signaling.

In conclusion, the present work proposes that the GLUT-mediated anchoring will offer a synergistic impact on the cell signaling by means of co-immobilization of D-glucose and EGF molecules targeting receptors on cell membrane.

(166) Environmental Cues for Skeletal Muscle Progenitor Cell Proliferation and Differentiation

Boonen K.J.M., Langelan M.L.P., Baaijens F.P.T., Bouten C.V.C., Post M.J.

Eindhoven University of Technology, Dept of Biomedical Engineering, Eindhoven, The Netherlands

Skeletal muscle is a highly structured, well organised tissue that produces force by contraction of differentiated, multinucleated muscle cells, or myofibres. Muscle cell differentiation from muscle precursor cells has been widely studied to obtain insight in processes of myogenesis and muscle regeneration, and to engineer skeletal muscle as a replacement tissue in the human body. Because of their effectiveness in regenerating large muscle defects *in vivo*, we investigate the use of satellite cell derived progenitor cells from muscle biopsies as a cell source for muscle tissue engineering. Despite their *in vivo* potency, however, the proliferative and myogenic capacity of satellite cells *in vitro* has been disappointing. We therefore concentrate on optimizing culture conditions to mimic the natural niche environment of the cells by providing biochemical and biophysical cues. In a well-defined 2D culture model system we systematically investigate the relative and combined contributions of, e.g. substrate stiffness and deformation, substrate coating, and changes in culture medium (e.g. serum-free), where the potential for myogenesis is evaluated from morphological and functional parameters using immunofluorescence microscopy, RT-PCR, and western blots. The model system provides for high-throughput and well-controlled measurements and can be expanded to 3D tissue culture conditions using biomaterials and/or other cell types.

(167) Erythropoietin Producing Cells for the Treatment of Renal Failure Induced Anemia

Egydio F., Moore P., Zhang Y., Atala A., Yoo J.J.

Wake Forest Institute of Regenerative Medicine, Winston-Salem, NC, United States of America

Introduction: Anemia is an inevitable outcome of chronic renal failure due to the kidney's decreased ability to produce erythropoietin (EPO). We investigated whether supplementation of erythropoietin producing cells would be a possible treatment option for renal failure induced anemia. We examined the feasibility of selecting and expanding erythropoietin producing cells for cell-based therapy.

Materials and Methods: Mice renal cells were culture expanded and characterized for EPO expression. The levels of EPO production was measured in the cells grown under normoxic and hypoxic conditions. Renal cells mixed in collagen gel were implanted subcutaneously in athymic mice followed by retrieval at 14 and 28 days after implantation for analyses.

Results and Discussion: The cultured renal cells expressed EPO at each subculture stage. Western Blot detected EPO protein in the kidney cells of all passages tested. ELISA Assay showed that the renal cells grown under hypoxic condition produced EPO. Histologically, the retrieved implants showed cell survival and tissue formation *in vivo*. Presence of EPO producing cells were confirmed using EPO specific antibodies.

Conclusions: These results demonstrate that EPO producing renal cells can be grown, stably express EPO in culture, and are able to form tissue *in vivo*. This study shows that EPO producing cells may be used as a potential treatment option for anemia caused by chronic renal failure.

(168) Establishment of an Effective Coculture System for Ligament-Bone Interface Tissue Engineering

Nayak B.K.P., Goh J.C.H., Toh S.L.

National University of Singapore (NUS), Singapore

Ligament-bone interface (enthesis) is a fibrocartilaginous organ that adds significant insertional strength to the interface and is highly resistant to avulsion forces. Thus, a tissue engineered construct of enthesis is more promising than ligament-alone construct to replace a severely injured ligament. We hypothesize that stem cells cocultured between ligament and bone cells would differentiate to fibrocartilage lineage under cyclical mechanical compression. But, the transmission of key chondrogenic factors and the mechanical stimuli across the cells require expression of gap-junctions at their lateral surfaces.

This study aims to establish an effective *in vitro* coculture system of bone marrow derived stem cells (BMSCs) and bone (osteocytes)/ligament cells, as shown by the expression of gap-junctional proteins. Ligament and bone cells were dual-stained, with DiI Red (cytoplasmic dye to differentiate bone/ligament cells from BMSCs), and Calcein-AM (gap-junction permeable dye). Two sets of coculture was established by mixing cell suspensions of ligament/bone cells with BMSCs (ratio 1:10) and culturing on petri-dishes. Expression of gap-junctions was studied by dye-transfer of Calcein from donor cells (dual-labelled ligament/bone cells) to recipient cells (unlabelled BMSCs) by fluorescence microscopy at 12 and 24-hrs post-coculture. This was further confirmed and quantified by FACS at 2, 6 and 12-hrs post-coculture. Significant transfer of Calcein into BMSCs was observed and FACS analyses showed a gradual increase with time in the percentage of BMSCs acquiring Calcein (Bone-BMSC: 2 hrs-4.4%, 6 hrs-10.4%, 12 hrs-14.2%; Ligament cell-BMSC: 2 hrs-4.3%, 6 hrs-9%, 12 hrs-13.8%). These results confirmed successful expression of functional gap-junctions between the stem cells and osteocytes/ligament cells *in vitro*. It will serve as a strong foundation to envisage our long term objective, that is, to tissue engineer

ligament-bone interface by establishing a trilineage coculture system.

(169) Evaluation of Chondrocyte Response to Electrospun Polymer-Ceramic Composite Scaffolds

Goodchild R.L.¹, Hatton P.V.¹, Ryan A.J.¹, Norton D.¹, Reaney I.M.¹, Migliaresi C.², Maniglio D.²

¹Centre for Biomaterials, University of Sheffield, South Yorkshire, S10 2TN, United Kingdom

²Department of Materials Engineering and Industrial Technologies, University of Trento, Via Mesiano 77, I-38050 TRENTO, Italy

Introduction: Incorporating ceramics into polymer scaffolds offers a route for the repair of osteochondral defects. The ceramic component is intended to act as an osteoconductive biomaterial for bone “anchoring” whilst the polymer component would support the tissue engineered cartilage.

Aim: The aim of this work was to evaluate the suitability of electrospun polymers and electrospun polymer-bioglass composites scaffolds for cartilage tissue engineering.

Materials and Methods: Melt-derived 45S5 bioglass was fabricated in-house (particle sizes of < 45 µm). Solutions of polystyrene (PS) 30% (w/w) in tetrahydrofuran and poly(D,L-lactic acid) (PDLLA) 14% (w/w) in chloroform were electrospun to produce scaffolds. 45S5 bioglass (10% w/w) was incorporated into a proportion of the scaffolds. Bovine primary chondrocytes (P2) were seeded onto the scaffolds and expanded for 7 days prior to 33 days differentiation. The scaffolds were examined using scanning electron microscopy (SEM) before and after cell seeding. Finally, the tissue engineered constructs were evaluated using histology and biochemistry.

Results: Chondrocyte proliferation was observed on both the polymer scaffolds and the polymer-ceramic composites. Histologically, there was little difference between matrix production on the scaffolds examined. SEM confirmed that cells were able to proliferate on all of the scaffolds. The presence of bioglass in the scaffold did not influence the histological appearance of tissue engineered cartilage.

Conclusions: Electrospun scaffolds supported chondrocyte proliferation and the development of a hyaline-like cartilage extracellular matrix.

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(170) Evaluation of the Biocompatible and Osteoinductive Properties of Calcium Phosphate Cement Incorporated with PLGA Microparticles

Link D.P., Van den Dolder J., Van den Beucken J.J.J.P., Cuijpers V.M., Wolke J.G.C., Jansen J.A.

Radboud University Nijmegen Medical Center, Department of Periodontology & Biomaterials, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

In this study, the biocompatibility and potential osteoinductive properties of calcium phosphate (CaP) cement incorporated with poly(D,L-lactic-co-glycolic) acid (PLGA) microparticles was evaluated in a subcutaneous implantation model in rats. Short-term biocompatibility was assessed using pure CaP discs and discs incorporated with PLGA microparticles (20% w/w), which were either or not preincubated (8 weeks) in water. Long-term biocompatibility and osteoinductivity was assessed using CaP discs with varying amounts (5, 10, or 20% w/w) and diameter sizes (small, 0–50 µm; medium, 51–100 µm, or large, 101–200 µm) of PLGA microparticles.

Samples were evaluated using histology, x-ray diffraction (XRD) and microcomputed tomography (µCT).

The short-term biocompatibility results showed only a mild tissue response for all implant formulations, irrespective of disc preincubation, during the early implantation periods of 2, 4, 8 and 12 days. Quantitative histological evaluation revealed that no significant differences in capsule and interface scores were present between the different formulations of implants at individual time points.

The results concerning long-term biocompatibility and osteoinductivity showed that all implants were surrounded by a connective tissue capsule containing fibroblasts and collagen bundles. The implants containing 20% PLGA with medium- and large-sized microparticles showed fibrous tissue ingrowth throughout the implants indicating PLGA degradation and interconnectivity of the pores. However, no bone formation was observed in both 12 and 24 week implants.

In summary, CaP/PLGA implants evoke only a minimal inflammatory response. In view of potential osteoinductive properties the various CaP/PLGA formulations with a different porosity did not induce bone formation in the current experimental design.

(171) Evidence for a Coupling Between Bone Formation and Scaffold Resorption in an Engineering Scaffold of Tricalcium Phosphate Bioceramic and Bone Marrow Stromal Cells

Mastrogiacomo M., Papadimitropoulos A., Cedola A., Peyrin F., Giannoni P., Pearce S.G., Alini M., Giannini C., Guagliardi A., Cancedda R.

Istituto Nazionale per la Ricerca sul Cancro, and Dipartimento di Oncologia, Biologia e Genetica dell'Università di Genova, Genova, Italy

Resorbable porous ceramic constructs, based on silicon-stabilized tricalcium phosphate, were implanted in critical-size defects of sheep tibias, either alone or after seeding with bone marrow stromal cells (BMSC). Only BMSC-loaded ceramics displayed a progressive scaffold resorption, coincident with new bone deposition. To investigate the coupled mechanisms of bone formation and scaffold resorption, X-ray computed microtomography with synchrotron radiation (microCT) was performed on BMSC-seeded ceramic cubes. These were analyzed before and after implantation in immunodeficient mice for 2 or 6 months. With increasing implantation time, scaffold thickness significantly decreased while bone thickness increased. The microCT data evidenced that all scaffolds showed a uniform density distribution before implantation. Areas of different segregated densities were instead observed, in the same scaffolds, once seeded with cells and implanted *in vivo*.

A detailed microX-ray diffraction analysis revealed that only in the contact areas between deposited bone and scaffold, the TCP component of the biomaterial decreased much faster than the HA component. This event did not occur at areas away from the bone surface, highlighting coupling and cell-dependency of the resorption and matrix deposition mechanisms. Moreover, in scaffolds implanted without cells, both the ceramic density and the TCP:HA ratio remained unchanged with respect to the pre-implantation analysis.

(172) *Ex Vivo* Expanded Endothelial Progenitor Cells (EPC) for the Vascularisation of Dermal Substitutes

Hendrickx B., Luttun A., Van den Berge S., Dickens S., Vranckx J.J.

KULeuven Laboratory of Plastic Surgery and Tissue Engineering Research, Herestraat 49, 3000 Leuven, Belgium

Objectives: The actual absence of a reliable autogenic full thickness skin equivalent hinders recovery and prospects on better quality of life in deep burns and skin disorders. For permanent integration and restoration of volume and elasticity, neovascularisation of skin substitutes is the missing link. We investigated the formation of a vascular network into distinct substrates by *ex vivo* expanded EPC.

Methods: Late outgrowth EPC were isolated from human and porcine peripheral blood by density gradient centrifugation. Obtained clones were characterised by RT-PCR and immunohistochemistry, and functionally by fibrin and matrigel tube forming and acLDL uptake assays. EPC were subsequently integrated in a commercially available dermal substitute (Integra), an in-house prepared human dermal decellularised matrix or a fibroblast multilayer to examine their angiogenic capacities *in vitro*.

Results/Perspectives: Porcine EPC clones were successfully obtained from three independent isolations at a frequency of 1 per 10⁷ PBMCs. Human clones were obtained from two donors at a frequency of 1 per 10⁸. Cells could be propagated for at least 25 population doublings. Porcine and human cells expressed VE-cadherin, KDR, CD34, CD31, eNOS and vWF in the same order of magnitude as PAECs and HUVECs, respectively. Importantly, expression of CD11b, a monocyte marker, was negligible. Cells behaved functionally like endothelial cells as evidenced by their capacity to take up acLDL and form tubes on matrigel. *In vivo* studies with dermal surrogates including these cells are being performed in nude athymic Balb/C mice to examine their effect on angiogenesis and wound healing.

(173) *Ex Vivo* Expansion of Human Umbilical Cord Blood Hematopoietic Stem/Progenitor with Support of Microencapsulated Rabbit Mesenchymal Stem Cells in Rotating Wall Vessel

Liu Y.¹, Liu T.Q.¹, Fan X.B.¹, Liu M.Y.¹, Zhao G.F.¹, Bao C.Y.¹, Ma X.H.¹, Cui Z.F.²

¹Dalian University of Technology, Dalian R&D Center for Stem Cell and Tissue Engineering, Dalian, 116023, China

²Oxford University, Oxford Centre for Tissue Engineering and Bioprocessing, Department of Engineering Science, Oxford OX1 3PJ, United Kingdom

Expansion of umbilical cord blood mononuclear cells (UCB MNCs) was carried out with the support of alginate-chitosan-alginate (ACA) microcapsules containing rabbit BM mesenchymal stem cells (MSCs) in a rotating wall vessel (RWV) bioreactor and culture plates in serum-free medium supplemented with conventional doses of purified recombinant human cytokines (SCF 50 ng/ml, FL 50 ng/ml, TPO 50 ng/ml and IL-3 25 ng/ml) for 7 days. The total nucleated cell density, pH and osmolality of the culture medium in the two coculture systems were measured every 24 hours. Flow cytometric assay for CD34+ cells and methylcellulose colony assays were carried out at 0 h, 72 h and 168 h. The pH and osmolality of the medium in the two coculture systems were maintained in the proper ranges for hematopoietic stem cells (HSCs) and progenitors. The RWV bioreactor, combined with a cell-dilution feeding protocol, was efficient to expand UCB MNCs. At the end of 168 h culture, the total nucleated cell number was multiplied by about 107-fold, and CD34+ cells 26-fold, and colony-forming units in culture (CFU-Cs) 19-fold. While in culture plates, however, total nucleated cell number was multiplied by 10-fold and CD34+ cells and CFU-Cs numbers changed mildly. It is demonstrated that the expansion of HSCs and progenitors can be achieved at a large-scale with the support of microencapsulated stromal cells in the RWV bioreactor.

Key words: stem cell expansion; UCB hematopoietic stem/progenitor cells; CD34+ cells *ex vivo* expansion; mesenchymal stem cell; ACA microcapsule; RWV bioreactor

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(174) *Ex Vivo* Expansion of Pericardial Patch Structures

Veggian P.¹, Sachweh J.S.², Flanagan T.C.¹, Koch S.¹, Tschoeke B.¹, Schmitz-Rode T.¹, Jockenhoevel S.¹

¹Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, Pauwelsstrasse 20, 52074 Aachen, Germany

²Department of Paediatric Cardiac Surgery, University Hospital, Pauwelsstrasse 30, 52074 Aachen, Germany

Pericardial patch structures play an important role in the reconstruction of congenital heart failures, e.g. correction of a univentricular heart (Fontan operation). The difficulty associated with Fontan tunnel reconstruction is the limited availability and size of autologous pericardium due to adhesion after the preliminary surgical procedure. The aim of the current project is to realise *ex vivo* cultivation and expansion of autologous pericardium.

The cultivation technique is based on a combination of a surgical mesh graft technique and principles of tissue engineering. By applying parallel incisions and uni-directional stretching, pores generated within the pericardial tissue are subsequently sealed with a fibrin gel/cell suspension. In order to stimulate the cell growth into the pores, a specific bioreactor system was developed to generate cyclic stress conditions. After production of a mechanically stable patch structure, the procedure of incision, sealing and cultivation is repeated until the tissue has reached the intended dimensions.

After the cultivation and expansion of meshed porcine pericardium for 10 days, a dense tissue was obtained inside the mesh. Histological analysis revealed synthesis of types I and III collagen,

and elastin, in approximate physiological relations. However, the neotissue was irregular and non-uniform, depending on the position in the bioreactor and the variable cyclic stress that is associated with the position.

This preliminary investigation demonstrates the possibility of *in vitro* expansion of autologous pericardium to provide sufficient tissue for reconstruction of congenital heart failures. Further modifications of the customised bioreactor will be undertaken to generate a more homogenous tissue structure.

(175) Examining Cell Guidance Using Proteomics and Microscopy

McNamara L.E.^{1,2}, Riehle M.O.¹, Dalby M.J.¹, Burchmore R.²

¹University of Glasgow, Centre for Cell Engineering, Joseph Black Building, Glasgow G12 8QQ, United Kingdom

²University of Glasgow, Sir Henry Wellcome Functional Genomics Facility, Joseph Black Building, Glasgow G12 8QQ, United Kingdom

Microarray studies indicate that surface topographies, such as microgrooves (Dalby et al, 2003), can have a considerable effect on cellular gene expression. This is not sufficient to infer changes at the protein level, however, and it is important to assess the correlation between topography and cell responses at the proteomic scale.

Recent evidence suggests that surface chemistry can impact on the protein profile of human macrophages (Dinnes et al, 2007), including changes in cytoskeletal protein levels, and it is likely that surface topography will also affect the cellular proteome. We are investigating the use of 2D gel electrophoresis and mass spectrometry to examine the effect of a microgrooved topography (groove depth 5 µm, 12.5 µm spacing) cast in PDMS (polydimethylsiloxane) on the proteome of hTERT BJ-1 human fibroblasts. We are concurrently examining the impact on cellular morphology and subcellular architecture. Initial results suggest that there is a substantive effect on cytoskeletal organisation, which can be quantitated by proteomic analysis.

References

1. Dalby, M.J., Riehle, M.O., Yarwood, S.J., Wilkinson, C.D.W., and Curtis, A.S.G. (2003). Nucleus alignment and cell signalling in fibroblasts: response to a micro-grooved topography. *Exp. Cell Res.* 284, 274-82.
2. Dinnes, D.L.M., Marçal, H., Mahler, S.M., Santerre, J.P., and Labow, R.S. (2007). Material surfaces affect the protein expression patterns of human macrophages: a proteomics approach. *J. Biomed. Mater. Res.* 80A, 895-908.

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(176) Experimental and Computational Characterization of Sponceram Supports

Sanz-Herrera J.A., Kasper C., Van Griensven M., García-Aznar J.M., Ochoa I., Doblaré M.

Group of Structural Mechanics and Materials Modelling, Aragon Institute of Engineering Research (I3A), University of Zaragoza, Spain

The experimental evidence of the dependence of cell proliferation and differentiation *in-vitro* on the mechanical environment aims to the need of the characterization of porous scaffolds in terms of mechanical and flow properties. In this sense, here we evaluate the Young's modulus and intrinsic permeability for three types of Sponceram[®] cell carriers developed for *in-vitro* applications. Young's modulus and ultimate compression stress were obtained performing a two-plate compression test carried out in a universal micro tester machine Instron[®] for several representative samples of each specimen. A permeability test is implemented in order to evaluate flow rate and pressure gradient, i.e., Darcy's law, in the linear range. Moreover, porosity and specific surface were estimated through micro-CT of the scaffold microstructure. The experimental data are compared with those obtained numerically over several representative volume elements (RVEs) of the scaffolds microstructure. Experimentally, results yield to a Young's modulus of 8.73 ± 4.64 , 11.03 ± 2.24 , 29.40 ± 1.21 MPa and the intrinsic permeability of $3.76 \text{ e-}8 \pm 4.38 \text{ e-}9$, $3.17 \text{ e-}8 \pm 3.62 \text{ e-}9$ and $1.79 \text{ e-}8 \pm 4.09 \text{ e-}9 \text{ m}^2$ for samples S20-90, S30-90 and S30-90 HA, respectively. Computational results show a good agreement with the experimental measured values.

(177) Experimental Characterisation and Computational Modelling of 2D Cell Spreading for Skeletal Regeneration

Sengers B.G., Please C.P., Oreffo R.O.C.

Bone & Joint Research Group, DOHAD, University of Southampton, Southampton General Hospital, Tremona Road, SO16 6YD, United Kingdom

Insufficient scaffold cell ingrowth is a key problem in tissue engineering and the clinical application of porous biomaterials for bone reconstruction. To address this issue, migration and proliferation of an interacting cell population can be studied in, for example, wound healing assays in 2D cultures. To derive intrinsic parameters that can be used for predictions, it is essential to generalise the results of these experiments by means of mathematical modelling. However, limited experimental observations impede a precise assessment of such theoretical models and insight in the underlying mechanisms. In the current study, experiments and image analysis methods were developed to provide a detailed spatial and temporal picture of how cell distributions evolve. These methods were applied to a circle migration assay to quantify migration as well as proliferation of skeletal cell types including human osteosarcoma cells (MG63) and Human Bone Marrow Stromal Cells (HBMSCs). The high level of detail with which the cell distributions were mapped enabled complete evaluation of the correspondence between experimental results and theoretical model predictions. This analysis revealed that the standard Fisher equation is appropriate for describing the migration behaviour of the HBMSC population, whereas for the MG63 cells a sharp front model is more appropriate. Information obtained from these studies has been translated into computational models to predict cell ingrowth in 3D scaffolds, which are currently under evaluation. In combination with experiments, this type of mathematical model

will prove useful in understanding and predicting cell ingrowth and improving strategies and control of skeletal tissue regeneration.

(178) Exploring Fluorescence as a Tool for Real-Time Monitoring of Scaffolds Degradation

Cunha-Reis C., Bagnaninchi P.O., El Haj A.J., Yang Y.

Institute for Science and Technology in Medicine, School of Medicine, Keele University, Thornburrow Drive, Hartshill, Stoke-on-Trent ST4 7QB, United Kingdom

Monitoring the degradation of Tissue Engineering scaffolds in response to various conditions stands as a major challenge. In this work we introduce an innovative monitoring model which enables real-time following up of scaffolds' breakdown, therefore constituting the framework for on-line monitoring. We have developed a model in which fluorescent molecules are incorporated in the scaffolds. As the polymer undergoes degradation the fluorescence intensity (FI) of the scaffolds decreases either by elimination of fluorophore molecules attached to the degraded fragments, or by quenching phenomena induced by the accumulation of degradation products, like H^+ ions, originating a stimuli responsive scaffold. The combination of several imaging and analytical techniques will enable the establishment of a correlation between FI and degradation status of scaffolds at the different stages of degradation. Ultimately, the degradation status at any given point will be assessed by simply quantifying the FI of the scaffolds.

Chitosan matrices labelled with TRITC were incubated in a lipase solution. The FI was quantified by confocal microscopy before and after incubation. After 4 days incubation the FI of the matrices was found decreasing 15%. This decrease was followed by a decrease in their weight and an increase in the FI, TRITC and reducing sugars content of the culture medium. The results clearly show a close relation between decrease in FI and degradation status of the matrices. Therefore this work stands as the first report of a consistent strategy for the development of a system capable of on-line monitoring the degradation of TE scaffolds.

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(179) Expression of Stem Cell Niche Markers in Cartilage

Barreto Henriksson H., Thornemo M., Jonsson M., Lindahl A.

Institute of Biomedicine, Göteborg University, Göteborg, Sweden

Aim: To study expression of stem cell niche markers in cartilage.

Introduction: In skin as well as in bone marrow it has been known for decades that there exist special stem cell niches, a structural unit where stem cells are located and recruited when needed. The wnt-catenin signalling pathways as well as BMP signalling related pathways are closely involved in the homeostasis as well as regulation of the cells within the stem cell niche. A panel of stem cell niche markers and their activities, C-kit, Frizzled, Patched, ABCG2, BMP1a, Noggin, beta-catenin and Notch1, were constructed using the dermal papilla and bulge region of hair fol-

licle in human skin as a positive control. The panel was applied on cartilage and chondrocytes from human and rabbit.

Methods: Immunohistochemistry using fluorescence tagged antibodies and Nikon Microphot FX, ACI software microscopy combined with Confocal techniques (Zeiss LSM 510 Meta) were used. RT-PCR on mRNA taken from monolayer cultures was done to study gene expression of the stem cell niche markers.

Results: Within the Pericondrial region of the rabbit knee a positive expression of the stem cell niche markers c-kit, Frizzled and ABCG2 were detected. Young human articular cartilage from extra digit expressed Frizzled, Patched, Notch1 and Bmp1a. Cultured chondrocytes from extra digit toe and adult cartilage also expressed niche markers. The protein expression of the respective gene was confirmed by RT-PCR.

Conclusion: Within the joint, the Pericondrial region demonstrates a stem cell niche architecture. Cartilage from human toe specimen as well as cultured chondrocytes expresses some of the stem cell niche markers. If these results represent the existence of a "true" stem cell niche within cartilage or Perichondrial region of the joint has to be further confirmed.

(180) Ex-Vivo Expansion of Umbilical Cord Blood-Derived Hematopoietic Stem Cells Supported with Autogenetic Mesenchymal Stem Cells Simultaneously

Fan X.¹, Liu T.¹, Ma X.¹, Cui Z.²

¹Dalian R&D Center for Stem Cell and Tissue Engineering, Dalian University of Technology, Dalian 116023, China

²Oxford Centre for Tissue Engineering and Bioprocessing, Department of Engineering Science, Oxford University, Oxford OX1 3PJ, United Kingdom

Autogenetic mesenchymal stem cells (MSCs) not only can support hematopoietic stem cells (HSCs) expansion *in vitro* with directive contact but also alleviates complications and leading to a speedy recovery of hematopoiesis during hematopoietic stem cell transplantation. In order to achieve this destination, expansion of HSCs with autogenetic MSCs simultaneously is required. In the present study, we investigated the feasibility of expanding UCB mononuclear cells (MNCs) with appearance of autogenetic MSCs in the 2-D system. The cells were cultured in 6-well plate with IMDM medium containing 12.5% horse serum and 12.5% fetal bovine serum, supported with the combination of cytokines, including SCF (50 ng/ml), TPO (15 ng/ml), FL (15 ng/ml), IL-3 (15 ng/ml), GM-CSF (5 ng/ml) and G-CSF (5 ng/ml), and cocultured with rabbit-derived MSCs encapsulated in the beads of calcium alginate-chitosan (AC). With the diluted-feeding protocol, the expansion of NCs could be up to 627.6 ± 74.5 -fold at day 14. While CD34+ cell and CFU-Cs could be expanded up to 76.9 ± 2.6 -fold and 27.9 ± 1.2 -fold respectively at day 9. At the same time, plenty of fibroblast-like cells appeared on the bottom of the well plate. By induction and flow cytometric analysis, the fibroblast-like cells could differentiate into osteoblasts, chondrocytes and adipocytes and expressed MSCs surface markers of CD29CD44 positive and CD34CD45 negative. After the analysis of orthogonal experiment

about culture influencing factors, we found the support of rabbit-MSCs encapsulated in AC beads and the addition of the combination of cytokines are the main factors to result in the significant expansion of HSCs.

(181) Fabrication of SFF-Based PLGA Scaffolds using a Micro-Deposition System

Kim J.Y., Seol Y.J., Cho D.W.

POSTECH, Dept. of Mechanical Engineering, Pohang, Gyungbuk, 790-784, Korea

Objectives: Recent reports indicate that the development of scaffolds that have controllable pore sizes using solid free-form fabrication (SFF) technology would further progress tissue engineering [1]. In this study, three-dimensional poly-lactic-co-glycolic acid (PLGA) scaffolds having uniform pore size were fabricated with line widths of 200 μm and 300 μm using a newly developed deposition system. Mesenchymal stem cells (MSCs) were then cultured on the scaffolds to investigate functionality by characterizing cell adhesion and proliferation behavior.

Methods: A newly developed micro-deposition system was employed to fabricate three-dimensional scaffolds based on SFF technology. This system allowed fabrication of repeatable 3D microstructures to a resolution of tens of micrometers. Fabrication of the PLGA scaffolds required the combination of several technologies, including three-axis motion control, thermal control, pneumatic control, and CAD/CAM software. PLGA (50,000–75,000 Mw) is a biodegradable scaffold material that is used in bone and cartilage regeneration. Scaffolds were fabricated at 250 kPa and 130°C, with the pore size and line width manipulated by controlling the speed of the XY stage. Stacked scaffolds were constructed using a layer-by-layer process to obtain an overall scaffold size of 3.4×3.4×3.4 mm³. Adhesion and proliferation characteristics of MSCs were assessed quantitatively using the MTS assay, and qualitatively by SEM.

Results and Discussion: This study demonstrated that a micro-deposition system can successfully fabricate three-dimensional PLGA scaffolds having desired pore size and line width.

Reference

1. T.B.F. Woodfield, *Biomaterials* 25, 2004, 4149–4161.

(182) Factors Influencing Angiogenic Processes in the Vascu-Plug Unit: Analysis of Neovascularisation

Hiebl B.¹, Cao Y.², Knedlitschek G.¹

¹Institute for Biological Interfaces, Forschungszentrum Karlsruhe, Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

²Microbiology and Tumor Biology Center, Karolinska Institute, S-171 77, Stockholm, Sweden

Angiogenesis is a complex multi-step process involving a multitude of soluble cell signals and cellular elements. The role of different individual and combinatorial angiogenic factors in promoting angiogenesis is being studied for further use in a composite structure

combining scaffolds, drug releasing nanoparticles and cells in a 3D arrangement as described for the EU project VascuPlug.

The effect of several growth factors has been systematically studied in various animal models. Special emphasis has been given to the study of synergistic effects of the angiogenic activity of FGF-2 and PDGF isoforms. Among all analysed proangiogenic factors PDGF-AB/BB and FGF-2 in combination have proven to result in the most valid induction of functional arterial blood vessels. Underlying mechanisms are currently being studied.

It is well established that monocytes/macrophages can stimulate the process of neovascularisation. We have therefore included this aspect to further improve vascular morphogenesis by the addition of monocytes in the composite structure taking into account the physiological conditions during the *in vitro* period of culturing as well as during implantation. Therefore, parameters like the adequate cell ratio (human microvascular endothelial cells/human peripheral blood monocytes) have been studied *in vitro* upon their effects on the induction of vascular morphogenesis. Our results indicate that the presence of monocytes/macrophages indeed affect the onset of angiogenesis in the *in vitro* setting. To mimic physiological conditions during the *in vitro* culturing period the ratio of endothelial cells/monocytes should not exceed 1/5 to 1/10 to result in angiogenic effects.

(183) Fascial Tissue Reconstruction Using Acellular Collagen Matrix

Eberli D.^{1,2}, Yoo J.J.¹, Atala A.¹

¹Institute for Regenerative Medicine, Wake Forest University School of Medicine, United States of America

²University of Zurich, Departement of Urology, Switzerland

Statement of purpose: Various materials have been proposed as possible substitutes for fascial reconstruction. An ideal biomaterial should be biocompatible, non-immunogenic, durable, and be able to promote formation of new tissue. Acellular collagen matrices have been used experimentally and clinically for several applications, including urethra and bladder reconstruction. We investigated the feasibility of using a porcine collagen-based matrix as an off-the-shelf biomaterial for fascial tissue repair in 3 different tissue systems.

Methods: Biocompatibility of the acellular matrix derived from porcine bladders was assessed using cell viability, MTT and apoptotic index. The acellular collagen matrices were used as a sling material for the treatment of incontinence, abdominal hernias and penile tunica reconstruction in 24 rabbits. The animals were evaluated up to 3 months after implantation.

Results/Discussion: The acellular matrix did not induce significant changes in cell viability, proliferation and apoptotic activity when compared with the controls. All animals survived without any untoward effects. The matrices remained intact at their respective implantation sites and demonstrated the maintenance of fascial tissue function. Histologically, there was only a minimal inflammatory response, which gradually decreased over time.

Conclusions: These results show that the acellular collagen matrix derived from porcine bladders is biocompatible, durable and safe

for use *in vivo*. This matrix may be used as an off-the-shelf biomaterial for sling operations, for penile reconstruction and for the treatment of abdominal hernias.

(184) Femtosecond Nonlinear Optical Assays for Monitoring of Cryopreserved Stem Cells

Trainor N., Cui Z.F., Triffitt J., Tirlapur U.K.

Oxford Centre for Tissue Engineering and Bioprocessing, Department of Engineering Science, University of Oxford, Oxford, OX1 3PJ, United Kingdom

Cryopreservation is an important means for the long-term storage of stem cells for use in basic research, tissue engineering applications and in clinical stem-cell based therapies.

We have investigated the efficacy of antioxidants as cryoprotecting agents and developed non-invasive assays based on non-linear optical imaging (NLOI) in conjunction with specific fluorescent probes to monitor cell morphology, viability, attachment, pH, calcium, mitochondrial membrane potential, proliferation and differentiation potential before and after cryopreservation of human mesenchymal stem cells (hMSCs).

Cells were seeded onto plastic coverslips in 12-well plates at a density of 3.2×10^5 cells/mL and cultured in 2 mL of a-MEM. For cryopreservation cells were treated with a freezing medium (90% FBS and 10% DMSO) supplemented with different concentrations of (a) butylated hydroxyanisole, (b) α -tocopherol, and (c) L-glutathione. Samples were frozen at a cooling rate of 5°C/min to 4°C, 1°C/min to -30°C, 2°C/min to -60°C and then transferred either to liquid nitrogen or into cryo-freezer. The samples were thawed in a 37°C incubator, freezing medium withdrawn and maintained for 6 hours in fresh a-MEM. At the end of which or after induction of differentiation cells were labelled with specific fluorescent probes and monitored by NLOI.

Herein, we demonstrate the use of (1) NLOI based optical assays as novel non-invasive means for vital 3D monitoring of morphological and physiological status of hMSCs pre- and post cryopreservation and provide evidence that (2) inclusion of antioxidants to the freezing medium improves viability, proliferation and morphogenetic potential of the hMSCs.

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(185) Fibrin Crosslink Stabilisation by Glutaraldehyde for Scaffold Manufacture

Di Y., Dye J.F.

RAFT Institute, Royal Free Hospital NHS Trust, Mount Vernon Hospital, Northwood, Middlesex, HA6 2R, United Kingdom

Fibrin has long history of use as a tissue adhesive and temporary scaffold for tissue regeneration, with renewed interest as an intrinsically pro-angiogenic biomaterial. However, susceptibility to proteolytic degradation and dissolution limits its use as a tissue-engineering scaffold. Although glutaraldehyde (GTA) is an established crosslinking agent for collagenous materials, its complex chemistry necessitates precise knowledge for safe and successful stabilisation of new fibrin-based scaffolds.

Fibrinogen film was used as a model to establish analytical methods and optimise crosslinking chemistry. Assay of conjugated Schiff-base GTA-protein adducts to measure crosslinking density was explored to optimise processing parameters. The data correlated with other crosslinking density determination methods (equilibrium swelling, free ϵ -NH₂ content & resistance to protease digestion). Differential scanning calorimetry measurement of denaturation temperature proved less successful for fibrinogen than collagen. Crosslinking density was highly dependent on GTA: protein mass ratio, showing a logarithmic increase to a maximum at 50% GTA:fibrinogen. Collagenous materials reacted with more GTA with less Schiff-base formation. In buffered ethanolic media at pH 7.4, used to control problematic protein leaching, optimal reaction time was found to be 4 hours at 22°C. In aqueous media, both reaction degree and rate were higher, but gave lower dimensional stability. Release of free GTA in physiological buffer over two weeks was measured by perchloric acid-phenol assay. Furthermore, NaBH₄ treatment reduced Schiff-base recovery and eliminated free GTA release; however, FTIR showed that this treatment affected the protein structure, thus necessitating optimisation of this process.

This study provides data for development of novel fibrin-based biomaterials.

(186) Fibrin Gel Scaffold Optimization for Tissue Engineering Vascular Grafts

Shaikh F.M.^{1,2}, Brien T.P.O.², Callanan A.², Kavanagh E.G.^{1,2}, Burke P.E.^{1,2}, Grace P.A.^{1,2}, Badylak S.F.³, McGloughlin T.M.²

¹Department of Surgery, Mid-Western Regional Hospital, Limerick, Ireland

²Centre for Applied Biomedical Engineering Research, University of Limerick, Limerick, Ireland

³Center for Pre-Clinical Tissue Engineering, McGowan Institute for Regenerative Medicine, Pittsburgh, Pennsylvania, United States of America

Introduction: Vascular tissue engineering offers a possibility of small diameter blood vessel substitutes. A major challenge is to find an optimum combination of biological and material properties to maintain structural viability. Xenogeneic extracellular matrix (ECM) materials have been used in numerous tissue engineering applications, but remain limited for some by their inherent mechanical and biologic properties. Fibrin gels have been shown to support angiogenesis and tissue repair but are mechanically too weak. The search for optimal cell infiltration and remodelling of ECM has lead the authors to examine the coating of xenogeneic matrix with fibrin gels.

Methods: ECM made from porcine urinary bladder membrane (UBM) was cut in circular pieces (1.5 cm²). Three scaffold types (UBM, Fibrin Gel and a UBM-Fibrin hybrid) were seeded with Human Umbilical Vein Endothelial Cells in 24 well plates to mimic biological infiltration. The scaffolds were placed in static culture for 24, 48 and 72 hours respectively. Various aspects of cellular growth were analyzed using optical and scanning electron microscopy and immunofluorescence.

Results: Optical and scanning electron microscopy demonstrated good cell and matrix integrity on the UBM-Fibrin Hybrid scaffold.

Immunofluorescence confirmed viable cells on all the three types of scaffolds. Higher cell seeding density resulted in more rapid creation of a confluent monolayer in all cases.

Conclusion: The results suggest that UBM Fibrin hybrid scaffold has a potential as a biocompatible natural acellular scaffold. An optimized hybrid scaffold needs to be developed.

(187) Fibroblast Growth Factor Receptors in Chondrogenesis

Hellingman C.A.¹, Kops N.², Koevoet W.J.¹, Frenz D.A.³, Baatenburg de Jong R.J.¹, Van Osch G.J.V.M.^{1,2}

¹Dept. Otorhinolaryngology & ²Dept. Orthopaedics, Erasmus MC, University Medical Center Rotterdam, The Netherlands

³Dept. Otolaryngology and Anatomy & Structural Biology, Albert Einstein College of Medicine, New York, United States of America

Introduction: In cartilage tissue engineering, control and stabilization of chondrocyte phenotype remains a problem. Therefore, better tools for monitoring and control are needed. It is known that Fibroblast Growth Factor Receptors (FGFR) play a role in embryonic chondrogenesis. During endochondral ossification FGFR2 expression in prechondrogenic condensation is followed by FGFR3 expression in differentiating chondrocytes and FGFR1 expression in hypertrophic cartilage. The aim of the present study was to investigate whether *in vitro* chondrogenesis of mesenchymal stem cells (MSC) shows a corresponding pattern in FGFR expression.

Materials and Methods: Bone marrow derived MSCs from 3 patients were cultured in pellets in chondrogenic medium. The pellets were harvested on different culture days for immunohistochemistry.

Results: Collagen II production is seen in all patients, beginning between the 2nd and the 3rd week, and seems to have a donor-specific localization within the pellet. Collagen X production follows at week 3-4 at sites where collagen II is present. At day 3 of culture, all FGFRs are expressed throughout the pellet. Expression of FGFR1 decreases the first 1-2 weeks specifically at locations where collagen type II is expressed later on, and rises again later, in hypertrophic chondrocytes. Expression of FGFR2 and 3 rises at similar location shortly before collagen II is expressed. FGFR4 is mostly expressed in hypertrophic chondrocytes.

Conclusion: During *in vitro* chondrogenesis FGFR1-4 are expressed in a spatio-temporal pattern comparable to embryonic endochondral ossification. With this knowledge we anticipate to find tools to monitor or control chondrogenesis in the future.

(188) Fibronectin Based Biomaterials as a Treatment for Spinal Cord Injury

Khatun F.¹, King V.¹, Alovskaya A.², Brown R.A.², Priestley J.V.¹

¹Neuroscience Centre, ICMS, St. Bartholomew's and the Royal London School of Medicine and Dentistry, Queen Mary University of London, 4 Newark Street, London, E1 2AT, United Kingdom

²University College London, Institute of Orthopaedics, Tissue Repair and Engineering Centre, RNOH Campus, Stanmore, HA7 4 LP, United Kingdom

Following spinal cord injury a series of secondary degenerative events occur that expand the area of initial damage and include the formation of large cavities within the spinal cord. We have begun to examine the use of injectable self-assembling fibronectin based biomaterials to improve recovery and reduce these secondary effects. Specifically, we have examined the effects of injecting a fibronectin/fibrinogen/thrombin (F/F/T) solution into the site of a spinal cord compression injury 1 week following the initial injury. At six weeks post-injury such treatment largely prevented the formation of cavities normally characteristic of this type of injury. In addition, animals treated with the F/F/T solution showed significantly greater recovery of hindlimb locomotor function compared to control animals. Immunohistochemical processing showed the presence of axons within the compression area of F/F/T including axons of primary sensory afferents as well as descending serotonergic bulbospinal axons. In addition, the compression site had extensive infiltration of Schwann cells and was highly vascularized. We also examined the suitability of F/F/T as a matrix for suspending cells within a lesion cavity. The results indicated that Schwann cells suspended within the FFT material and injected into a lesion cavity within the spinal cord survived for at least one week and had growing axons associated with them. Taken together, these results indicate that the F/F/T solution may not only be neuroprotective following spinal cord injury but may also be useful as a matrix for cell based therapies in the central nervous system.

(189) Finite Element Modelling of a Cell Stressing Device

Milnes P.¹, Southgate J.², Walker D.C.³, Jin Z-M.¹

¹University of Leeds, Department of Mechanical Engineering, Leeds, LS2 9JT, United Kingdom

²University of York, Jack Birch Unit of Molecular Carcinogenesis, Department of Biology, York, YO10 5YW, United Kingdom

³University of Sheffield, Kroto Institute, Department of Computer Science, Sheffield, S3 7HQ, United Kingdom

The FlexerCell is a commercial device for subjecting cell cultures to regimes of mechanical strain. The cells are cultured on a flexible membrane, which is drawn taut over a fixed post to induce strain. A finite element model of the membrane and post was created such that system artefacts were assessed under both ideal and non-ideal operating conditions. Analysis of the model was used to determine the effects of inadequate lubrication and the effects of repositioning the central support post. Model results were compared to static measurements observed on sample FlexerCell plates.

The results indicated that when used in accordance with the manufacturer's recommendations, a region of uniform strain existed over the area of the post, however, outside this area >2 fold strain fields were generated. Lubrication between membrane and post was necessary, as lack of lubrication eliminated all strain over the central post region. Under lubricated conditions the required strain was limited to the area immediately over the post. The system was tolerant to post offset, with 0.5 mm offset producing less than 3% variation in strain.

Over the central area of the membrane, the prediction agreed with the observed strains and with the literature [1]. Users of the

system should, however, be aware of potential artefacts incurred from population-based sample analysis, as up to 64% of cells will receive an inappropriate strain.

Reference

1. Vande-Geest et al. *J.BioMech.* 37, pp 1923–8.

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(190) Finite Element Study of Regular Scaffolds for Bone Tissue Engineering

Lacroix D., Villagomez M., Planell J.A.

Institute for Bioengineering of Catalonia (IBEC), Universitat Politècnica de Catalunya, Diagonal 647, 08028 Barcelona, Spain

Perfusion bioreactor has been shown to be beneficial for 3D cell culture. However, little is known on which are the optimal parameters of shear stress and scaffold architecture supporting cell differentiation into chondrogenic and osteoblastic lineages. A structural model of 3D scaffolds of regular geometry was developed, with the aim of studying the influence of mechanical stimuli on cell seeding and tissue development, and the development of design criteria for scaffold architecture for bone engineering. Repeated truncated octahedron and hexagonal prism units were selected with various strut thicknesses and cell diameters. Solid and fluid finite element analyses were performed to investigate cell architecture on mechanical stimuli. Scaffolds of same porosity showed different compressive stiffness which could influence cell attachment, proliferation and differentiation. Moreover, it is predicted that longitudinal struts are mainly loaded in compression whereas radial struts are mainly loaded in tension. This could also have some influence in cell behaviour. This study shows that numerical modelling is a useful tool for the design of scaffolds for tissue engineering. Scaffolds of identical porosity but different stiffness can be modelled; the effect of mechanical stimuli predicted by computer model and can be compared with *in vitro* studies.

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(191) From Strong Human Tissue-Engineered Vascular Grafts to an *In-Vivo* Porcine Model

Stekelenburg M., Pullens P.A.A., Post M.J., Baaijens F.P.T

Eindhoven University of Technology, Department of Biomedical Engineering, Eindhoven, The Netherlands

Vascular tissue engineering represents a promising approach for the development of living small diameter blood vessels that can be used for replacement therapy. Our group recently succeeded in culturing strong human vascular grafts based on a fast-degrading polymer scaffold (PGA/P4HB) and fibrin gel. Tubular constructs were seeded with venous myofibroblasts and subjected to dynamic strain conditions. The resulting grafts demonstrated burst pressures of 900 mmHg after 4 weeks of *in-vitro* culture. In the physiologically relevant range, the mechanical properties of the grafts, assessed by tensile testing, were similar to that of native arteries. A

next step involves *in-vivo* implantation in an animal model, this will further elucidate the potential of the engineered grafts.

Porcine grafts will be cultured using myofibroblasts harvested from jugular veins of pigs. The first step involves adapting the human tissue-engineering protocol to the use of porcine cells. This step is not trivial as it is known that cells from (young) animals behave differently compared to human cells from older patients, which were used to culture the human vascular grafts. Current experiments show that the culture of porcine grafts displaying similar mechanical behavior as the engineered human grafts will require, e.g., shorter culture times and less serum in the culture medium.

(192) Functional Enhancement of Bioreactor Assisted Engineered Skeletal Muscle

Zare S., Christ G., Moon D.G., Zhao W., Atala A., Yoo J.J.

Institute for Regenerative Medicine, Wake Forest University School of Medicine, United States of America

The inability to engineer clinically relevant functional muscle tissues remains a major hurdle to successful skeletal muscle reconstructive procedures. We have previously demonstrated that muscle cell seeded scaffolds exposed to a constant biomechanical stimulation achieved enhanced cellular organization. This goal of this study was to determine whether contractile function could be further enhanced by fusing individual muscle cells to form myofibers prior to mechanical stimulation.

Primary human muscle precursor cells (MPCs) were placed in a muscle differentiation medium to induce cell fusion. MPCs were then seeded onto collagen-based acellular tissue scaffolds with matrigel coating (1.0×0.3×0.3 cm), and subjected to cyclic strain in a bioreactor system. The computer-controlled bioreactor was programmed to exert ±10–20% of the cell-seeded scaffold at a frequency of 3 times per minute for the first 5 minutes of every hour.

Differentiated single muscle cells fused and formed multinucleated myofibrils in culture. Bioreactor stimulation of differentiated engineered muscle produced viable tissue with appropriate cellular fusion and organization. Scanning electron microscopy and histology of the bioreactor stimulated muscle tissue showed uniform myofiber attachment and orientation on the scaffolds, with expression of muscle specific markers. Electrophysiological studies revealed important differences in excitation-contraction coupling in these cells; only the current-voltage relationships in the differentiated skeletal muscle cells were affected by addition of acetylcholine. These findings suggest that differentiation of muscle cells prior to the bioreactor stimulation may further enhance muscle tissue function *in vivo*, by more closely mimicking the physiology of native muscle.

(193) Functional Tissue-Engineering of the Urinary Bladder

Bolland F.¹, Korossis S.², Ingham E.², Fisher J.², Kearney J.N.³, Southgate J.¹

¹Jack Birch Unit of Molecular Carcinogenesis, Dept Biology, University of York, Heslington, York, YO10 5YW, United Kingdom

²Biomedical Engineering Research Centre, School of Mechanical Engineering, University of Leeds, Leeds LS2 9JT, United Kingdom
³Tissue Services, National Blood Service, 6 Estuary Banks, Speke, Liverpool L24 8RB, United Kingdom

Reconstruction of the urinary bladder is required for patients with chronic incontinence and cancer. As physical stimuli may modulate tissue development, the aim of this study was to produce functional bladder tissue constructs by re-seeding decellularised bladder matrices under appropriate biomechanical conditions.

A bioreactor system incorporating continuous circulation of culture medium was designed to simulate the physiological filling/voiding action of the bladder by delivering cyclic biaxial strain fields. Validation with fresh bladder tissue produced variable results with inconsistent retention of the bladder urothelium and evidence of apoptotic and inflammatory cells within the stromal tissue.

Full-thickness porcine bladders were decellularised by sequential expansion and incubation in hypotonic buffers containing SDS and nuclease enzymes and characterised by histological and bio-mechanical techniques [1]. The bladder matrix was completely acellular, but retained native bladder histoarchitecture, with no change in the ultimate tensile strength. The decellularised tissue was seeded with human bladder stromal cells and cultured statically or with mechanical stimulation. The extent of cell attachment and migration was determined by histology. The matrix was bio-compatible as cells began to infiltrate after 14–21 days in culture.

We have developed a novel acellular matrix and bioreactor system that, with further optimisation, can be used to study bladder tissue development, including paracrine interactions between urothelial and stromal compartments, cell-matrix interactions and the role of mechanical forces on tissue functionality.

Reference

1. Bolland F., Korossis S., Wilshaw S.P., Ingham E., Fisher J., Kearney J.N., Southgate J. 2007. Development and characterisation of a full-thickness acellular porcine bladder matrix for tissue engineering. *Biomaterials*. 28(6):1061–70.

(194) Gene Expression in Stem Cells Following Stimulation Using Magnetic Particle Technology

Sura H.S., Magnay J., Dobson J., El-Haj A.J.

Institute of Science and Technology in Medicine, Keele University, Thornburrow Drive, Hartshill, Stoke-on-Trent, Staffordshire, ST4 7QB, United Kingdom

Stem cell therapy has been proposed as a strategy for tissue repair and regeneration, but it has been difficult to reproducibly control the differentiation potential of the cells *in vitro* and *in vivo*. The current study has demonstrated a remote targeting approach for controlled cell differentiation along different lineages.

Stem cells were transfected with Ca²⁺ channel, or TREK-1 antibody coated particles (ø 250 µm, Nanomod). Transfected cells with internalised particles were seeded in 6-well plates in 2 ml of complete DMEM and were exposed to a 1 Hz cyclical loading in a vertical force magnetic bioreactor for one hour every other day for one week. Following the treatment, cells were harvested in a lysis buffer, prepared for real-time PCR analysis, and a bank of osteogenic and chondrogenic gene assays were performed.

This study has shown that following treatment using our bioreactor model, osteogenic and chondrogenic gene expression was significantly different with cells that were transfected with particles coated with TREK-1 antibody when compared with the Ca²⁺ channel antibody coated particles and blank controls. It also suggests that it may be possible to specifically target sites on stem cells in order to activate ion channels and enhance differentiation of the cells along a desired lineage using magnetic particle tagging.

(195) Gene Expression Profiling of Fibroblasts in Response to Applied Bio-mechanical Force

Eastwood M.¹, Shiwen X.¹, Bou-Gharios G.³, Sarraf C.¹, Black C.², Abraham D.²

¹Centre for Tissue Engineering Research, University of Westminster, 115 New Cavendish St., London W1W 6UM, United Kingdom

²Department of Rheumatology, Royal Free Hospital, London, NW3 2PF, United Kingdom

³Muscle Cell Biology Group, MRC Clinical Science Centre, ICSM, Hammersmith Campus, London W12 0NN, United Kingdom

Introduction: The repair process involves the synthesis of new connective tissue components and significant changes in the bio-mechanical forces to which the fibroblasts are exposed. These changes in the 'environment' provoke fundamental alterations in fibroblast function and metabolism. We have used fibroblast populated three-dimensional collagen lattices and the application of precise mechanical loading regimes to recapitulate bio-mechanical stimulation *in vitro* and to assess the resultant changes in global fibroblast gene expression.

Methods: Fibroblast populated collagen lattices were cast and either directly processed for RNA analysis or subjected to mechanical loading by attachment to a tensioning-Culture Force Monitor. Matrices were then subjected to the following regimes. A. Constitutive contraction for 24 hrs; or B. Constitutive contraction for 12 hrs followed by 6 hrs of cyclical loading. FPCL were homogenized in Triazol, biotinylated cRNA probes generated and expression profiles assessed by hybridization to Gene chips (Affymetrix). Data were analysed and differential expression defined as greater than 3.5 fold induction.

Results: Examination of expression profiles revealed that of the ~12,000 sequences on the gene chip approximately 4,000 were expressed in all fibroblast samples examined. Of these 4,000 genes, almost 10% were found to be differentially expressed between control FPCL and lattices that had undergone a contraction regime. Gene sequences found to be significantly elevated included proteases (serine protease), structural proteins (myosin, smoothelin), growth factors (VEGF), transcription factors (c-fos), extracellular matrix modifying genes (TIMP-3, PAI-1) and receptors (PAR).

Discussion: Transcriptional profiling yielded a large number of differentially expressed gene sequences when comparing fibroblasts cultured in collagen lattices under distinct mechanical loading regimes. This data suggest that bio-mechanical stress is not only a potent modulator of gene expression but can also provoke specific changes in fibroblast transcriptional profiles in response to differing external mechanical stimuli.

(196) Gene Expression Profiling of Human Mesenchymal Stem Cells Activated by SDF1- α

Stich S.¹, Haag M.¹, Kaps C.², Ringe J.^{1,2}, Sittinger M.¹

¹Tissue Engineering Laboratory, Department of Rheumatology, Charité-University Medicine Berlin, Tucholskystr. 2, 10117 Berlin, Germany

²TransTissue Technologies GmbH, Tucholskystr. 2, 10117 Berlin, Germany

Objective: In recent years it was demonstrated that mesenchymal stem cells (MSC) are able to migrate according to stimulation with chemotactic cytokines (chemokines). Their migration potential is a premise for the use of MSC for *in situ* tissue engineering applications. The aim of this ongoing study is to characterize the molecular signaling network, which underlies the SDF1- α stimulation of human MSC.

Methods and Results: The isolation of human bone marrow-derived MSC was performed according to standard protocols using density gradient centrifugation. Cell-morphology was analyzed during culture expansion in medium containing 10% FBS. Furthermore, FACS analysis of MSC-relevant surface markers SH-2, SH-3, CD14 and CD34 was performed. The chemotactic effect of SDF1- α on MSC was measured in a 96-well Boyden Chamber assay. MSC showed a dose-dependent chemotactic effect for SDF1- α , the ligand for CXCR4. Moreover, using microarray technology, gene expression profiles of MSC, activated for 20 h with medium containing SDF1- α , were compared to controls without SDF1- α . Bioinformatics using Genesis software for cluster analysis and Ingenuity software for pathway analysis are ongoing. Differentially expressed genes in controls and SDF1- α activated cells and evaluation of underlying signalling networks will be presented.

Discussion: Here, for the first time, the signaling network of SDF1- α stimulated MSC is demonstrated. These data provide basic insights into the gene regulation of chemokine activated MSC, which are prominent candidates for *in situ* tissue engineering applications.

(197) Gene Expression Profiling of Human Mesenchymal Stem Cells Chondrogenic Induced in Pellet Cultures and in 3D Composites

Ringe J.¹, Greber M.¹, Dehne T.¹, Noël D.², Delorme B.³, Mrugala D.², Jorgensen C.², Charbord P.³, Häupl T.¹

¹Charité-University Medicine Berlin, Tissue Engineering Laboratory and BCRT, Tucholskystr.2, 10117 Berlin, Germany

²Inserm, U844, 80 Avenue Augustine Fliche, Montpellier, F-37032, France

³Inserm, ESPRI EA3855, 10 bld Tonnelles, Tours, F-37032, France

Introduction: Usually the chondrogenic potential of molecules is tested in pellet cultures. Drawbacks are a weak reproducibility, difficult RNA isolation and the duration of 4 weeks. Our aim was to compare the pellet assay with a 3D culture assay, based on fibrinogen and PGLA, on the molecular level.

Methods: Human bone marrow MSC ($n = 3$ donors) were TGF β 3 induced in pellet assays (2.5×10^5 cells). MSC from 3 independent donors were embedded in fibrinogen/PGLA and TGF β 3 stimulated. RNA from native cartilage, pellet cultures (day 0, 7, 21) and 3D grafts (week 0, 2, 4) was used for gene expression profiling with Affymetrix HG-U133 plus 2.0 chips. Standardized tools for cluster analysis and pathway analysis were used for bioinformatics.

Results: Hierarchical clustering resulted in 3 main groups. The pellet group could be discriminated from a group containing the grafts and cartilage. The latter group is further divided into the cartilage and graft group. By day 21, Sox9 and type II collagen expression in pellets was 6% and 1% of GAPDH respectively, in day 14 composites 17% and 150%, and in cartilage 27% and 49%. Pairwise comparison with undifferentiated MSC revealed an increased expression in more than 80% of the comparisons for type II collagen and COMP in pellets, Sox9, type II, IX, XI collagen, aggrecan and COMP in grafts, and Sox9, type II, IX collagen, aggrecan and COMP in cartilage. Bioinformatic analysis is ongoing.

Conclusion: 3D grafts showed more similarities to cartilage than pellets. Therefore, and because of their easy handling and short duration time, they represent a good alternative for the standard assay.

(198) Gene Expression with Smart Polycationic Macromolecules

Pişkin E.¹, Türk M.², Dinçer S.³

¹Hacettepe University, Chemical Engineering Department and Bioengineering Division, Ankara, Turkey

²Kırıkkale University, Department of Biology, Kırıkkale, Turkey

³Yıldız Technical University, Bioengineering Department, Istanbul, Turkey

In this study, *in vitro* and *in vivo* studies of temperature sensitive, polycationic poly(N-isopropylacrylamide) and polyethyleneimine copolymers (poly(NIPA/PEI25L)) (1) were performed. Copolymer and copolymer/plasmid DNA complexes were positively charged as +7.6 and +12.8, respectively. Gel retardation assay confirmed good complex formation and release of plasmid DNA in response to temperature and pH. In cytotoxicity tests, the smooth muscle cell (SMC) viability was over 80%. The uptake of the complexes by SMCs were quite high, however the best gene expression efficiency achieved with the copolymeric vectors was about 30% with the complex prepared with a polymer/plasmid ratio 6. Gene expression efficiency was enhanced up to 50% by changing the temperature from 37°C to 28°C. Preliminary *in vivo* studies were performed above and below LCST in lung, heart, liver, kidney, muscle and also subcutaneous in 5-week old mice. Gene expression ratio was higher in lung, tibial muscle, and subcutaneously than other tissues (heart, liver and kidney) above LCST. Then, temperature decrease caused an increase in gene expression amount in tibial muscle and subcutaneously that was revealing of the contribution of temperature-sensitivity on DNA release and gene expression.

Reference

1. Türk M, Dinçer S, Yuluğ I G, Pişkin E. *in vitro* Transfection of HeLa Cells with Temperature Sensitive Polycationic Copolymers. J Control Rel 2004; 96; 325–340.

(199) Gene Expressions in Tendon Fascicles Subjected to Cyclic Tensile Strain

Maeda E., Shelton J.C., Bader D.L., Lee D.A.

Queen Mary, University of London, Medical Engineering Division and IRC in Biomedical Materials, School of Engineering and Material Science, Mile End Road, London, E1 4NS, United Kingdom

Mechanical loading is essential for the homeostasis of load-bearing biological tissues. It has been shown *in vitro*, using rat tail tendon fascicles, that cyclic tensile strain inhibits proteinase production and upregulates collagen synthesis. However, the mechanisms underlying these mechanosensitive responses are remained unknown. We hypothesised that the expression of anabolic and catabolic genes in tenocytes is regulated with cyclic strain in a duration-dependent manner. Fascicles dissected from rat tail tendon were incubated in DMEM + 10% FBS. Cyclic tensile strain (3% magnitude superimposed on 2% static strain, 1 Hz) was applied for 10 minutes, 1, 6, or 24 hours. Each experiment was carried out along with unstrained control fascicles. Both strained and unstrained samples were collected at the end of each incubation period. Expression levels of messenger RNAs in both strained and unstrained samples were firstly normalised to 18s rRNA expression level, and the ratio of the expression level of strained samples to that of unstrained control samples was determined for each gene at each time point. Type I collagen expression levels were upregulated after 10 minutes or 1 hour of cyclic strain, but returned to unstrained level after 6 hours of straining. Type III collagen was upregulated by 1 hour cyclic strain. Matrix metalloproteinases 3 and 13 expression levels in strained samples were inhibited throughout the 24 hour strain period when compared to unstrained samples.

(200) Generating Bone Forming Scaffolds with a Cell Guidance Micro-Network

Keeney M., Damodaran G., Pandit A.

National Centre for Biomedical Engineering and Science, National University of Ireland, Galway, Ireland

In an idealised scaffold design, tethered biomolecules will aid cellular infiltration and will promote neovascular network. It is hypothesised that the precursor for cellular infiltration is the existence of a structural micro-network. The objective of this research is to design and characterise a scaffold with an internal network for guided cellular infiltration. Collagen/Calcium Phosphate (Coll/CaP) scaffolds were prepared under physiological conditions [2] and patterned with micro channels via protrusion of 300 µm needles through an outlined template. Coll/CaP interaction and structure were analyzed via Fourier Transform Infrared Spectroscopy (FTIR), Energy Dispersive X-Ray Analysis (EDX) and X-Ray Diffraction (XRD). Micro-CT data ($n = 4$) were acquired and analysed through 3D reconstruction software to characterise the porous network. Mechanical properties were obtained from compression testing. FTIR analysis showed the growth of CaP crystals on the collagen network via stretching of a phosphate peak (10^{18} cm^{-1}) while binding was found between Ca^{2+} ions on the CaP and -COO- on collagen [2]. XRD analysis indicated synthetic hydroxyapatite

while EDX determined the calcium to phosphate ratio to be 1.42, hence it is likely that synthetic hydroxyapatite and a more degradable form of CaP co-exist. [1] Image analysis found surface area to increase by over 300% with pore spacing ranging from 400–600 µm. In conclusion a Coll/CaP scaffold with a designed micro-network has been fabricated and characterised for chemical and physical architecture.

References

1. Bradt et al. (1999) J. Chem. Mater., 11, pp. 2694–2701.
2. Kikuchi et al. (2001) Biomaterials, 22, pp. 1705–1711.

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(201) Generation of Vascularized Distal Pulmonary Tissue Constructs *In Vitro* and *In Vivo*

Lelkes P.I., Mondrinos M.J., Koutzaki S., Finck C.M.

Drexel University, School of Biomedical Engineering (PIL and MJM) and Department of Surgery (SK,CMF), Philadelphia, PA 19104, United States of America

We hypothesize that engineering functional lung tissue for transplantation will create a potential treatment modality for numerous pediatric and adult lung diseases. We recently described how to engineer 3-D pulmonary tissue constructs *in vitro* using primary isolates of fetal mouse distal lung cells containing epithelial, mesenchymal and endothelial cells (Mondrinos et al., Tissue Engineering, 12:717–728, 2006). Over a 7 day time period, the mixed cell population in these hydrogel-based constructs organized into alveolar forming units (AFUs): sacculated structures, which, in terms of morphology and cytodifferentiation, were reminiscent of native distal lung. By using a unique, serum-free medium supplemented with a cocktail of tissue-specific growth factors, we induced concomitant alveolization and neovascularization when culturing the cells both in hydrogels or 3-D electrospun scaffolds made of natural proteins, but not in scaffolds composed of synthetic polymers. Our data suggest that our *in vitro* model is capable of recapitulating the parallel morphogenesis of epithelial and endothelial pulmonary tissue components, which may occur through dynamic paracrine interactions. In a mouse model *in vivo*, incorporation of the primary lung cell isolates into Matrigel plugs, implanted either s.c., under the kidney capsule or in the lung, lead to the formation of sacculated AFUs in close proximity to patent capillaries. Effective functional vascularization, however, was only observed upon addition of angiogenic growth factors to the scaffolds and their controlled release over time. Importantly the use of a fluorescent cell tracker confirmed that the neovessels in the constructs were comprised of endothelial cells from both the host and the grafts. These data demonstrate that it is feasible to generate vascularized pulmonary tissue constructs *in vivo* with proper epithelial differentiation.

(202) Genetic Manipulation of Human Mesenchymal Progenitors to Promote Chondrogenesis Within Polysaccharide Templates

Babister J.C., Tare R.S., Green D.W., Inglis S., Oreffo R.O.C.

Bone & Joint Research Group, Developmental Origins of Health and Disease, University of Southampton, Southampton, SO16 6YD, United Kingdom

Given the paucity of clinically viable cartilage formation regimes we have examined a non-viral gene delivery and tissue engineering approach, whereby Sox-9 transfected human mesenchymal progenitors were encapsulated within alginate/chitosan capsules to promote chondrogenesis.

Human bone marrow stromal cells and articular chondrocytes were transfected with flag-tagged Sox-9 plasmid and encapsulated within alginate/chitosan templates. Samples were also encapsulated with un-transfected cells and cells transfected with the empty vector pcDNA. Constructs were placed into Synthecon rotating-wall bioreactors, held in static conditions or examined *in vivo* using the sub-cutaneous implant model in SCID mice for 28 days.

Sox-9 transfection was demonstrated after 24 hours by western blot analysis using antibodies for anti-flag Sox-9 and anti-Sox-9. After only 7 days *in vitro* bioreactor and static culture, regions of cell-generated cartilaginous matrix were demonstrated in alginate samples containing Sox-9 transfected cells, as demonstrated by alcian blue staining and Sox-9 immunohistochemistry. After 28 days *in vitro* and *in vivo*, samples encapsulated with Sox-9 transfected cells demonstrated regions of cartilaginous matrix comprising approximately 25% of the construct, as confirmed by alcian blue staining, Sox-9 and type-II collagen immunohistochemistry, which was absent in samples encapsulated with un-transfected cells. Extracted protein from *in vivo* constructs indicated expression of Sox-9 and type-II collagen in Sox-9 transfected constructs that was absent in un-transfected cells.

Cartilage-like matrix formation was significantly increased in Sox-9 constructs confirming the presence of Sox-9 transfected cells promotes chondrogenesis. This has significant implications for strategies to regenerate cartilage and, ultimately, future therapies for human articular cartilage defects.

(203) GFP Expressing Cell Line as Screening Tool for Biomaterials

Engelhardt E., Aibibu D., Gries T., Hilborn J., Adam M., Wurm F.

Laboratory of Cellular Biotechnology, EPFL, Lausanne, Switzerland

Synthetic biomaterials play an important role in regenerative medicine and tissue engineering. They are asked to be non-toxic, non-immunogenic and to support cell proliferation and cell growth. Materials designed to mimic the natural environment of cells better and to achieve higher cell attachment and cell proliferation have to be tested for their suitability in cell culture. We have developed a fast and sensitive screening system for biomaterials based on a CHO cell line stably expressing the green fluorescent protein (GFP). The measurement of GFP-specific fluorescence allows determining cell growth and cell attachment on the biomaterial whether cells are growing on the surface or inside the material. GFP-specific fluorescence is directly related to the number of cells. This assay gives the opportunity to compare different materials as well as samples made of the same material but having different physical parameters in a fast and sensitive way. Preliminary data showed that higher polymer thickness and volume generally fa-

voured cell attachment. Moreover, it seemed that lowering liquid absorption ability and liquid storage capacity could enhance cell attachment on polymers.

(204) Glycosaminoglycan Synthesis by Mesenchymal Stem Cells in Response to Stretch

New S.E.P., Chester A.H., Yacoub M.H., Taylor P.M.

Imperial College London—NHLI, Heart Science Centre, Harefield, United Kingdom

Introduction: The synthesis of appropriate extracellular matrix (ECM) by cells in tissue engineered heart valve constructs will be vital for its ability to function and maintain integrity *in vivo*. We have investigated the synthesis of glycosaminoglycans (GAGs) by mesenchymal stem cells (MSCs) in response to cyclic force.

Method: Human mesenchymal stem cells were seeded on collagen type I membranes and subjected to 14% stretch at 0.6 Hz for 4 days ($n = 3$) using a Flexercell FX4000 system. Total sulphated-GAGs (s-GAGs) were determined using the Blyscan assay and the effect of stretch on GAGs and proteoglycans (PGs), as well as any phenotypic changes, were examined using immunofluorescence staining.

Results: The total amount of s-GAGs from the non-stretched group (1.3 ± 0.3 ng/ng protein) was 45.4% higher than the stretched (0.59 ± 0.06 ng/ng protein), although the difference was not statistically significant. Immunofluorescence showed morphological changes for the GAGs and PGs when stretched, the greatest being for biglycan.

Conclusion: Previous work has shown s-GAG content to be significantly reduced in stretched aortic valve leaflets when compared to non-stretched (1). Our data show that MSCs react in the same way, decreasing or inhibiting s-GAG production in response to stretch. These results have important implications for the use of MSCs in tissue engineering heart valves, as well as expanding our knowledge regarding the role of PGs and GAGs.

Reference

1. Balachandran K., Konduri S., Sucusky P., Jo H., Yoganathan A.P. (2006) *Ann Biomed Eng* 34:1655–65.

(205) Growth of NG108-15 Cells and Schwann Cells on Solvent Cast PCL Films

Sun M.¹, Armstrong S.J.², Kingham P.J.², Terenghi G.², Downes S.¹

¹Materials Science Centre, Department of Engineering and Physical Sciences, The University of Manchester, United Kingdom

²Blond McIndoe Research Laboratories, The University of Manchester, United Kingdom

Reconstruction of peripheral nerve defects over short distances (10–20 mm) could benefit from the development of novel biomaterials to bridge the gap and encourage nerve regeneration. Bioresorbable materials represent an alternative clinical application to the currently favoured repair method of autograft. The properties

and synthesis of the polymers require specific material and surface properties with *in vitro* biocompatibility with nerve cells. Here, we report a study in which we have produced microporous poly (ϵ -caprolactone) (PCL) films by solvent casting, and assessed their suitability as nerve guidance tubes. The synthetic scaffolds were fabricated using dichloromethane (DCM). The polymers had pore sizes between 3–10 microns in diameter and 1–4 microns in depth. The thickness of the films (0.024 ± 0.0027 mm) was adjustable by changing the concentrations of the casting solution. Partial hydrolysis of the polymer with NaOH introduced random scissoring, which rendered the material more hydrophilic and therefore more biocompatible with neural cells. DCM-PCL films supported the attachment and proliferation of NG108-15 motor neuron-like cells and Schwann cells. Both cell types readily attached to the surface of the films and the MTS assay showed NG108-15 cells multiplied by approximately 80 fold and Schwann cell 35 fold after 8 days cultured on NaOH-treated DCM-PCL films. Differentiation of NG108-15 cells with dibutyl-cAMP induced the growth of extended neurites across the surface of the PCL films. As a synthetic, controllable product, PCL overcomes the limitations of animal-derived products and these *in vitro* results demonstrate that this material is a potential candidate for use in peripheral nerve repair.

(206) Growth of Rapid Proliferating Cells on Alginate Scaffold

Pokrywcznska M., Drewa T., Jundzill A.

Tissue Engineering Department, Chair of Medical Biology, Collegium Medicum of Nicolaus Copernicus University, Bydgoszcz, Poland

Background: Alginate is widely used in tissue engineering. The aim of this study was evaluation of alginate as a scaffolds for 3D culture of rapid proliferating cells.

Materials and Methods: In this study murine 3T3 cell fibroblast line was used. 3T3 cells were cultivated in DMEM/Ham's F-12 (PAA) medium supplemented with 10% FBS (PAA) and antibiotics (Penicillin, Streptomycin, Amphotericin B) at 37°C and atmosphere of 5% CO₂.

Cells in density of 2, 4, 6 million/ml were mixed with 1% (w/v) middle viscosity alginate (Sigma). One ml aliquots of the cell suspensions was gelled by CaCl₂ (102 mM). Cells were cultured in alginate scaffold for 30 days at 37°C and atmosphere of 5% CO₂. The medium was changed every two days. Histopathologic examination by hematoxyline and eosin staining in the end of experiment was done. Morphology of cells suspended in alginate under the inverted microscope was observed. Photographic documentation was done.

Results: After a week of culture small colonies within the alginate scaffold were observed. After two weeks of culture cell migrated from the inner part of scaffold and formed large microspheres. No scaffold covered by cell layers was obtained. Migration of the rapid proliferating cells from 3D scaffold was observed. Cells were detached from dishes two times during experiment.

Conclusion: Alginate is not a good biomaterial for durable implant. Using alginate as a scaffold for transplantation of cells with high proliferation rate carries a risk of cells migration.

(207) Heart Tissue Engineering Using a Novel Elastomer and ES-Derived Cardiac Cells

Chena Q.Z., Wright J.S., Harding S.E., Junaid S., Hansen U., Jawad H., Boccaccini A.R., Ali N.N.

Imperial College London, Department of Materials, National Heart and Lung Institute, and Department of Mechanical Engineering, London SW7 2AZ, United Kingdom

Heart disease remains the leading cause of death and disability in industrialised nations. The common end-point of cardiac diseases is congestive heart failure (CHF) (or just heart failure), a condition in which the heart cannot pump a sufficient amount of blood to the body. The most common precipitating cause is myocardial infarction. Eventually, heart transplantation is the final treatment option to end-stage heart failure. Owing to the lack of organ donors and complications associated with immune suppressive treatments, however, scientists and surgeons constantly look for new strategies to repair the injured heart. One approach is to use a heart patch to deliver embryonic stem cell-derived cardiomyocytes to the infarcted areas. In the present work, the heart patch was produced with a novel elastomer, poly(glycerol sebacate) (PGS). This soft elastomer was synthesised at 120°C by polycondensation of glycerol and sebacic acid, with a mole ratio being 1:1. The produced PGS exhibited mechanical compatibility with heart muscle, Young's modulus being in the range of 0.04–0.07 MPa which is equal to the values of healthy heart muscles during the normal beating process. Biodegradation assessment in a simulated body fluid and cell culture medium showed that the present PGS is biodegradable. Initial *in vitro* cell culture work showed that PGS was biocompatible, with the embryonic stem cell-derived cardiomyocytes beating up to one and half months when cultured on the synthesised PGS. In conclusion, PGS is shown.

(208) Hepatocytes Co-cultured with Bone Marrow Stromal Cells Help in Maintenance of Hepatocyte Functions

Chang S.C.N., Lin T.M., Chung H.Y., Jeng L.B.

Department of Plastic Surgery, China Medical University Hospital, Taichung, Taiwan

Humans are subject to exposure to acrolein, which is also a metabolite of the anticancer drug cyclophosphamide. Allyl alcohol (AA) causes a periportal-specific hepatocellular lesion in animal livers. Liver injury is dependent upon AA's metabolism to the reactive aldehyde acrolein via alcohol dehydrogenase.

Bone marrow comprising heterogeneous cell populations contains certain progenitors to differentiate into multiple mesenchymal cell lineages. To identify differentiation plasticity of adult bone marrow mesenchymal stem cells (MSCs) into hepatocyte-like phenotypes, we used a co-culture model.

There are different concentrations in AA to treat rabbit hepatocyte alone and co-culture of MSC/AA treated hepatocyte. The controls were performed without AA. After 10 days of co-culture with injured liver cells, MSC expressed specific markers for hepatocytes by RT-PCR and Western blot. We tested the parameters in MTT assay, the maintenance of urea synthesis and another factor in the maintenance of albumin secretion.

The effectiveness of AA in causing death of hepatocytes can be deduced, through the production of a dose-response curve. MSCs exhibited hepatocyte-like phenotypes after co-cultivation with liver cells. There are reduced in hepatocyte functions also found in the co-culture system with proportionate to the MTT assays.

This data evidenced that the guided hepatic differentiation of MSC is proportionate to the activity of co-cultured hepatocytes. The hepatic environment is crucial to MSC differentiation. The presented evidence indicated the trans-differentiation potential of MSC developing to the hepatocytes. Future therapeutic application in hepatic regeneration will focus on the created niches to mimic the survival of hepatocytes *in vivo*.

(209) Heterogeneity of Hair Follicle Primary Cultures

Joachimiak R.¹, Drewa T.^{1,2}, Kaznica A.¹, Lysik J.^{3,4}

¹Tissue Engineering Department, Chair of Medical Biology, CM UMK, Bydgoszcz, Poland

²Chair and Department of Urology, CM UMK, Bydgoszcz, Poland

³Chair and Department of Bradytherapy and Oncology, CM UMK, Bydgoszcz, Poland

⁴Department of Pathology, Oncology Center, Bydgoszcz, Poland

Background: The hair follicle bulge is an important niche of adult stem cells. The aim of this study was to test the heterogeneity of hair follicle primary cultures.

Materials and Methods: 200 vibrissae were cut out from 3 month old Wistar male rats which were sacrificed in other experiments. Ethical committee agreement was obtained. Follicles were initially digested 14 h in dispase (Sigma, Germany) or collagenase (Sigma, Germany) and then 30 min. in trypsin (PAA, Austria). Two different DMEM (Sigma, Germany) based culture media were used: one supplemented with KGF (10 ng/ml; Amgen, USA) and the second with EGF (10 ng/ml; Sigma, Germany). Primary cultures were established and observed until 16th passage. Immunocytochemistry staining (anti-Pancytokeratine, CloneMMF; anti-HMB45 and anti-CD34, Dako, Denmark) and fotodocumentation were done.

Results: Primary culture established after dispase treatment expressed epithelial-like morphology during first three weeks in EGF supplemented medium. These cells changed into fibroblast-like cells, but still strongly expressed epithelial marker (cytokeratine) until 16th passage. These cells were CD34(+). Cells obtained after dispase digestion and cultivated in KGF supplemented medium also showed epithelial-like stable phenotype. These cultures were CD34(–). Fibroblast-like cell growth was observed after collagenase treatment in both KGF and EGF supplemented media. Cells from KGF supplemented media were HMB45(+).

Conclusion: Different culture media and digestion methods influenced primary cultures diversity. Primary culture from hair follicles contained keratinocytes and melanocytes. There is a suspicion that hair follicle cultures contained mesenchymal stem cells.

(210) Hollow Fiber Membrane to Reduce Necrosis in Tissue Engineered Constructs

Narasimha M.S.B., Rivron N.C., Rouwkema J., Stamatialis D., Van Blitterswijk C.A., Wessling M.

University of Twente Faculty Membrane Technology Group (MTG)
PO Box 217, 7500 AE, Enschede, The Netherlands

The arterial branching of blood vessels in the tissue of the body plays a critical role to supply nutrients and remove waste. The development of vascularization within the tissue engineered (TE) constructs remains a challenge.

Generally TE scaffolds have porous structure wherein cells migrate to form tissue. The major problem in such constructs is the nutrient supply to the core, as the proliferating cells themselves act as barrier for mass transfer. To address this problem of mass transfer limitation (necrosis), scaffolds integrated with porous hollow fibers (HF) are engineered. These HF mimic the arterial circulatory system for the *in-vitro* cultured tissue by supplying nutrients through the hollow fiber lumen at the inlet and removing waste at the outlet. As the fiber wall acts as a permeable barrier between the cell and the nutrient, high flux of the fiber are used to feed the proliferating cells without any shear damage to the cells.

In this work, polymeric hollow fibers are fabricated based on phase inversion technique using bio-compatible and bio-degradable polymers. Various fiber spinning parameters are studied to develop porous interconnected fibers. Experiments with HF for clean water flux and nutrient diffusion through the fiber with different porous morphology ranging from 50–80% are evaluated. In-vitro cell culture experiments are carried out using human bone marrow mesenchymal stem cells (H-BMSC) to study the seeding efficiency and proliferation.

(211) Host's Response Controls Tumor Formation Following Implants of Mouse Mesenchymal Stem Cells Seeded in a Bioscaffold

Tasso R., Postiglione F., Carida M., Cancedda R., Pennesi G.

Dept of Oncology, Biology, and Genetics, University of Genova, and National Institute for Cancer Research, Largo Rosanna Benzi 10, 16132 Genova, Italy

It has been recently described that mouse mesenchymal stem cells (MSC) can *in vitro* undergo tumorigenic process or to induce formation of sarcomas when injected in immunocompromised mice. We, however, lack information on whether MSC tumorigenicity can be controlled by the immune reaction mounted by a fully immunocompetent recipient. We argued that a strong immune response by the host could restrain MSC tumorigenicity, and we tested our hypotheses implanting combinations of MSC isolated from C57Bl/10 mice (H2b haplotype) and seeded on hydroxyapatite (HA) scaffolds into A) syngeneic recipients; B) allogeneic recipients (Balb/c mice-H2d haplotype) without immunosuppressant therapy; C) immunoincompetent animals (CD-nu/nu or NOD/SCID). We predicted a strong host's response in MSC/HA combinations implanted in allogeneic mice, while a minimal or no response is expected in the other two conditions.

We found that fibrosarcomas began to be evident in 10/12 (83%) of the syngeneic implants at 8 weeks after implantation; none (0/20) of the allogeneic implants showed tumor formation following them up to 16 weeks post-implant (syngeneic implants vs allogeneic implants $p=0.000001$); 8/10 (80%) of implants grafted in immunocompromised mice showed tumor formation starting from 8 weeks post-implantation (syngeneic implants vs implants in immunocompromised host $p=n.s.$; allogeneic implants vs implants in

immunocompromised host $p = 0.000007$). Fibrosarcomas invaded the implant surrounding tissues and did not allow the formation of an organized bone tissue within the bioscaffold.

We concluded that a strong allospecific immune response by an immunocompetent host could control tumor following implant of murine MSC seeded in a bioscaffold.

(212) Human Adipose Derived Mesenchymal Stem Cells (ADMSC) in Gelforcel® Hydrogel for Cartilage Repair: In Vitro Evaluation

Gindraux F., Barnouin L., Laganier L.

TBF, Dept Research and Development, 6 rue d'Italie, 69500 Bron, France

Introduction: Agarose alginate hydrogel (Gelforcel®) was used successfully as a matrix for autologous chondrocyte implantation. It presents interesting scaffold characteristics for tissue engineering: 3D environment allowing differentiation, stabilisation and homogenous distribution of the cells. The potential of the ADMSC included in this hydrogel instead of chondrocytes was evaluated *in vitro* in this work.

Materials and Methods: Cells are extracted from adipose tissue and then cultivated at different initial plating density in a plastic culture flask in expansion medium (containing of 10% calf bovin serum and 1ng/ml fibroblast growth factor 2). After 1 week, cultivated ADMSC were analyzed for phenotypic studies by flow cytometry with detection of specific ADMSC markers (CD73, CD90, CD105). Functionality was studied by: (1) clonogenic tests: Quantification of CFU-f (colony forming unit cells), (2) phenotype tests: culture of ADMSC included in gel in chondrogenic medium. After 3 weeks, gels were analyzed by immunohistochemistry (aggrecan and collagen II).

Results: Optimal plating density correspond to 10.103 ADMSC/cm². ADMSC phenotype was positive for CD73, CD90, CD105 markers. Expanded ADMSC presented a high clonogenicity at least equivalent to bone marrow (CFU number). ADMSC included in hydrogel and incubated with chondrogenic medium showed type II collagen and aggrecan expression.

Conclusion: The use of ADMSC included in Gelforcel shows *in vitro* promising results which would make possible to avoid the articular biopsy.

(213) Human Amniotic Fluid-Derived Stem Cells: A Novel Source of Dopaminergic Neurons?

Santos C., Klorig D., Chun S.Y., Atala A. and Soker S.

Wake Forest Institute of Regenerative Medicine, Wake Forest University Health Sciences, Winston-Salem, NC, United States of America

Current treatment for Parkinson's disease (PD) includes combination L-DOPA and carbidopa. However, within 5–10 years, this treatment becomes ineffective. The aim of our study is to investigate whether human amniotic fluid derived stem cells (hAFSC)

can be used for regenerative applications in neurodegenerative diseases like PD.

To yield dopaminergic cells, hAFSC were cultured in a 2 stage culture media systems that include growth factors to mimic the normal midbrain dopaminergic development. At different time points during differentiation, differentiated cells were analyzed using RT-PCR and immunostaining. At the end of differentiation the amount of cellular dopamine quantified and a whole-cell voltage clamp recording was performed.

The hAFSC at end of the expansion phase expressed nestin and Nurr1 and their expression was enhanced by exposure to hypoxic culture condition. At the end of terminal differentiation, cells look bipolar and pyramidal and showed expression of Girk2. After recording the inward currents, barium was added to the bath to block the Girk-specific inward current. A reduction in the amplitude of the currents evoked by the same hyperpolarizing voltage step protocol in the presence of barium indicated the presence of Girk-mediated potassium channel. Terminally differentiated cells secreted dopamine in a dose response manner, yielding approximately 2 nM/100,000 cells.

In this study we showed that hAFSC can be successfully differentiated into dopamine secreting neuron-like cells that express neuronal genes. The current study provided convincing preliminary data to suggest that hAFSC can be an alternative source of stem cells for transplantation surgery in patients with PD.

(214) Human Aneurysm as a New Source for (cardiac) Primitive Cells

Rubina K.A.¹, Tsokolaeva Z.I.², Melikhova V.S.², Tkachuk V.A.¹, Parfyonova Ye V.²

¹Department of Biological and Medical Chemistry, Faculty of Basic Medicine, Moscow State University, Moscow, Russian Federation

²Laboratory of Angiogenesis, Institute of Experimental Cardiology, Cardiology Research Center of Russia, Moscow, Russian Federation

Cardio-vascular diseases (myocardial infarction, MI) still lead the list of most dangerous diseases in modern world. Traditional treatments are coming to infarction consequences liquidation and prevention of repeated cases. Aneurysm formation is one of the most frequent complications after MI.

Recently, many papers have been dedicated to stem cell therapy in post-infarctional treatments and regeneration of human hearts with adult stem cells using bone marrow derived mesenchymal cells, stromal cells from adipose tissue and cardiac primitive cells. These cells are self-renewing, multipotential and positive to certain stem cell markers. Here we report that human aneurysm tissue could be the source of autologous adult stem cells. Aneurysm tissue could be isolated during the coronary artery bypass grafting operation. Immunohistochemistry and immunofluorescent staining of aneurysm tissue samples show that there are c-kit positive cells, which could be successfully isolated and used for *in vitro* expansion. Isolated and cultured cells contain subpopulations of c-kit (CD117), CD105, C-met, TroponinI, Connexin-43, Nkx-2.5, VEGF-R1, SDF-1 and CXCR-4-positive cells, which potentially implicate them in myocardial protection and regeneration. Using immunomagnetic sorting we isolated and expanded c-kit positive cells and showed that these cells are able to differentiate

into endothelial and neuronal cell lineages, expressing CD31 and β III-tubulin, correspondingly. This data suggest there are primitive c-kit cells present in aneurysm tissue which may represent a new source of primitive and multipotent progenitors for cardiovascular research and therapy.

(215) Human Bone Marrow Stromal Cell Growth on PLA-NanoHA Porous Scaffolds

Bloom K.¹, Qiao X.¹, Shakesheff K.M.², Wood D.J.¹, Yang X.B.¹

¹Department of Oral Biology, Leeds Dental Institute, Leeds LS2 9LU, United Kingdom

²School of Pharmacy, University of Nottingham, Nottingham NG7 2RD, United Kingdom

Introduction: The ability to develop biomimetic biomaterials for bone regeneration is a major challenge in orthopaedics and dentistry. Hydroxyapatite (HA) is the main inorganic component of natural bone with excellent biocompatibility and bioactivity. It has been widely used in clinics. However, its brittleness and fatigue-failure limit its application. In contrast, poly (L-lactic acid) (PLA) has better thermal plasticity and is biodegradable. However, PLA lacks bioactivity and toughness. Thus, a PLA and nano-scale HA composite may overcome these limitations to produce a bone substitute for clinical application.

Aim: The aim of this study was to investigate the effect of PLA-nanoHA composite porous scaffold on human bone marrow stromal cell (HBMSC) growth.

Methods: PLA and nano-scale HA were used to produce PLA-nanoHA composite porous scaffolds using supercritical fluid processing methods. HBMSCs were grown on PLA-nanoHA and PLA porous scaffolds in basal or osteogenic media for up to 6 weeks. Cell attachment, viability and proliferation were monitored by live/dead fluorescent markers using confocal microscopy and scanning electron microscopy. HBMSC differentiation was confirmed by histology.

Results: Enhanced HBMSC attachment and proliferation was observed on PLA-nanoHA scaffolds. Confocal microscopy showed better cell ingrowth in the PLA-nanoHA group. Histological staining showed that PLA-nanoHA group promoted HBMSC proliferation and differentiation confirmed by enhanced alkaline phosphatase expression compare to PLA alone.

Conclusion: This study showed that addition of nanoscale hydroxyapatite into a PLA scaffold, produced using the supercritical fluid method, enhanced the differentiation of HBMSCs along an osteogenic lineage.

(216) Human Cartilage Regeneration in an *In-Vivo* Mouse Model

Schueller G., Tichy B., Jagersberger T., Van Griensven M., Marlovits S., Redl H.

Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria

Dept. Trauma Surgery, Medical University, Vienna, Austria
Cluster for Tissue Regeneration, Vienna, Austria

Animal models examining cartilage regeneration methods with animal cells have the major limitation in the biological difference of human and animal cartilage. Therefore we propose an *in-vivo* model of human chondrocytes in a human cartilage acute defect environment.

Human full-thickness (2–4 mm) articular cartilage discs, 10 mm in diameter and attached to 3–6 mm of subchondral bone, were obtained from human femur heads upon joint replacement. Chondral defects (8 mm) were set without violating the subchondral bone. Human chondrocytes were isolated and cultivated for up to 3 passages. The cells were applied to the defect as a suspension of 10^7 cells/ml. The completely filled defect was covered with a thin sheet of human periosteum, fixed with a drop of fibrin sealant after 45 minutes adherence time. Discs were implanted subcutaneously in the back of nude mouse for 5 and 8 weeks.

Histological evaluation revealed a gradient of differentiation from the cartilage lateral side to the center of the defect. A proteoglycan rich matrix was formed with some chondron-like structures at the cartilage surface, which enlarged after 8 weeks compared to 5 weeks implementing a progress of cartilage regeneration. To offer the cells a larger cartilage surface we filled the defect with devitalised human cartilage powder showing a differentiating all over the defect. Discs implanted without cells or cover showed no chondrogenesis.

The introduced model is a promising new *in vivo* model to study human cell behaviour in a human cartilage defect environment, with the limitation of lacking synovial fluid and mechanical loading.

(217) Human Hair Derived Keratins Facilitate Regeneration of Peripheral Nerves *In Vivo*

Sierpinski P.¹, Garrett J.², Ma J.², Apel P.², Smith T.², Atala A.¹, Koman L.A.², Van Dyke M.¹

¹The Wake Forest Institute for Regenerative Medicine, and ²The Department of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston Salem, NC 27157, United States of America

Nerve defects commonly result from peripheral nerve injury and present a significant clinical challenge. Implantation of nerve guidance conduits containing tissue engineered scaffolds can enhance regeneration. However, no optimal material has been identified and clinically translated. The goal of this study was to further investigate the utility of novel keratin biomaterials to enhance peripheral nerve repair.

Keratin fractions were isolated from human hair, converted to a powdered form and re-hydrated to form keratin hydrogels. A 4 mm tibial nerve defect was produced in adult mice. A keratin-filled nerve conduit was then surgically implanted, with empty silicone conduits or autografts run in parallel. Six weeks later regeneration was apparent in empty, keratin-hydrogel filled conduits and autograft. Regeneration through the keratin gel resulted in the development of blood vessels grossly visible within the conduit. Electrophysiological studies revealed that the latency (in msec) was 1.76 in the keratin treated group vs. 2.10 in the empty and 2.26

in the autograft group. The nerve impulse amplitude (in mV) was 9.5 in the keratin group vs. 4.0 and 7.5 in the empty and autograft groups, respectively. Muscle force demonstrated comparable muscle reinnervation in all treatment groups. Histological analysis confirmed that the keratin hydrogel increased vasculature in nerve diameter.

The data demonstrate that insertion of keratin hydrogels into a nerve guidance conduit demonstrated a high degree of regeneration in a mouse model as confirmed by functional testing and histological analysis. Presumably keratin extracts do so via facilitating Schwann cell proliferation, migration, and adhesion *in vitro*.

(218) Human Outgrowth Endothelial Cells in Co-cultures with Osteoblastic Cells Form Microvessel-like Structures

Fuchs S., Malenica B., Kirkpatrick C.J.

Institute of Pathology, Johannes Gutenberg University, Mainz, Germany

Outgrowth endothelial cells from human peripheral blood cell cultures are a homogenous population of endothelial cells for potential applications in autologous cell therapies.

In this study we established 2-D and 3-D co-cultures of OEC with osteoblasts (primary and MG63) relevant for bone regeneration. We investigated the effects of the co-culture on the cellular organization of OEC with potential implications for the process of angiogenesis in complex tissue engineered constructs.

Human OEC were isolated, expanded and characterized as previously described (1, 2). MG63 and primary human osteoblasts were labelled with Cell Tracker to identify them in the co-cultures. Co-cultures were grown on fibronectin-coated Thermanox® or glass coverslips and assessed by confocal microscopy for endothelial markers, and by SEM and TEM for ultra-structural elements. Stability of the endothelial population under different culture conditions and after different time points was assessed by flow-cytometry. Using a rotating culture vessel system, co-cultures of OEC and primary osteoblasts were further established under 3-D conditions.

In co-cultures OEC formed in contrast to HUVEC highly organized microvessel-like structures, which were not affected by factors for osteogenic differentiation. TEM revealed lumen formation of OEC co-cultured with MG63 and primary osteoblasts with tight junctional complexes at endothelial cell contacts. In the 3-D complexes with human primary osteoblasts OEC formed an endothelial network identified immunohistochemically using CD31. We conclude that OEC provide a valuable source of autologous cells for vascularization of engineered tissues such as bone.

References

1. S. Fuchs, et al. 2006, Biomaterials 27, 5399–408.
2. S. Fuchs, et al. 2006, Cell Tissue Res 326, 79–92.

(219) Human Umbilical Cord Perivascular Cells (HUCPVCs) as Source of Mesenchymal Progenitors for Central Nervous System Regenerative Medicine: Role of Paracrine Factors and Direct Cell-to-Cell Contacts in Neurons/Glial Cells Viability, Proliferation and Differentiation

Salgado A.J.¹, Fraga J.S.¹, Neves N.M.^{2,3}, Reis R.L.^{2,3}, Sousa N.¹

¹Life and Health Science Research Institute, School of Health Sciences, University of Minho, Braga, Portugal

²3B's Research Group—Biomaterials, Biodegradable and Biomimetics, Department of Polymer Engineering, University of Minho, Braga, Portugal

³IBB, Institute of Bioengineering and Biotechnology, PT Associated Lab, Braga, Portugal

Umbilical Cord Stem/Progenitor Cells are able to integrate within the CNS upon injury and to improve the condition of animals suffering from neurodegenerative diseases. However the mechanisms by which such phenomena are mediated are unknown. Therefore the main objective of the present work was to understand how a population of umbilical cord progenitor cells isolated from the Wharton Jelly (HUCPVCs), regulates viability, proliferation and differentiation of post-natal hippocampal neurons and cortical glial cells. Hippocampal neurons and glial cells were exposed to HUCPVCs conditioned mediums (CMs) (obtained 24, 48, 72 and 96 after 3 days of culture of HUCPVCs) for 1 week. Direct contact co-culture systems were set by using neuron/glial cells feeder layers, on top of which HUCPVCs were plated. Cell viability and proliferation was assessed by MTS test and total protein quantification. Immunocytochemistry against GFAP (astrocytes), CD11b (microglia), O4 (oligodendrocytes) and HNA (HUCPVCs) was also performed. Cell viability and proliferation experiments revealed that HUCPVCs CM obtained for all time points did not cause any deleterious effects on both cell populations when compared to the control condition. Immunocytochemistry and total cell counts revealed that HUCPVCs CM triggered an upregulation of the proliferation on astrocytes, oligodendrocytes and differentiation of hippocampal neurons. For the latter this was noticed even in the absence of neuronal supplements B27 and FGF-2. Furthermore the co-culture systems revealed that glial cells were inducing HUCPVCs death, attributed to direct cell-to-cell contacts and cell proliferation dependent mechanisms. Noteworthy to mention is that the number of astrocytes and oligodendrocytes increased in the co-culture system, even when HUCPVCs were dying. We believe that the phenomena here in described are related to the release of specific growth factors by HUCPVCs. Future work will focus on the identification of the growth factors involved and consequent crosstalking mechanisms related to the phenomena herein presented.

(220) Hyaluronan-Based Biomaterial Modulates Catabolic Factors Expressed by Human Chondrocytes and Mesenchymal Stem Cells

Lisignoli G., Grigolo B., Manferdini C., Codeluppi K., Cavallo C., Desando G., Facchini A.

Laboratorio di Immunologia e Genetica, Istituti Ortopedici Rizzoli, Via di Barbiano 1/10, 40136 Bologna, Italy

Hyaluronan (HA) is the major non-protein glycosaminoglycan component of extra-cellular matrix (ECM) involved in cell positioning, proliferation, differentiation, as well as, in receptor-mediated changes in gene expression. Human chondrocytes and mesenchymal stem cells (h-MSCs) are widely studied for cartilage tissue regeneration since the homeostasis of this tissue in pathological condition is modulated by metalloproteinases (MMP-1, MMP-13) and inflammatory chemokine (CXCL8). We investigated whether an HA-based

biomaterial, already used as scaffold for autologous chondrocyte transplantation, could create also an environment which favours the down-regulation of catabolic factors expression. Human chondrocytes and h-MSCs were seeded on biomaterial and analyzed at 1, 7, 14 and 21 days after seeding. We found that in human chondrocytes the expression of CD44, well known HA receptor, increased over time showing the highest positivity at day 21 while in h-MSCs was highly expressed but not modulated by the biomaterial. Decreased levels of metalloproteinases (MMP-1, MMP-13) were observed both in chondrocytes and h-MSCs grown onto the HA-based scaffold. This was also confirmed by Real-Time PCR analysis which showed that the cells express the specific differentiated phenotype and in the meantime down-regulate the expression of these catabolic molecules. Moreover, we found that CXCL8 was down-modulated during chondrogenesis.

Our data clearly demonstrate that HA in this three-dimensional conformation acts as a signalling molecule for chondrocytes and h-MSCs down-regulating some catabolic pathways. The ability to reduce the expression and production of molecules involved in cartilage degenerative processes suggests its use also in the transplantation as therapeutic strategy to treat early lesions in osteoarthritic patients.

(221) Hydrodynamic Evaluation of a Bioreactor for Tissue-engineering Heart Valves

Bowles C.T., Van Loon R., Dreger S.A., Biglino G., Chan C., Parker K.H., Chester A.H., Taylor P.M., Yacoub M.H.

Heart Science Centre Harefield, National Heart and Lung Institute, Imperial College London, United Kingdom

Development of a tissue-engineered heart valve may be achieved by subjecting cell-seeded collagen scaffolds to mechanical cues. The production of a clinically effective tissue-engineered valve will therefore be dependent upon the determination and reproducible implementation of an appropriate conditioning regime in a pulsatile bioreactor.

We have designed and manufactured an autoclavable bioreactor, which circulates tissue culture medium within a reservoir/Windkessel circuit. The system consists of a computer controlled and electrically actuated, hydraulic, pulsatile pump to drive a ventricular assist device (VAD) with an inlet and outlet valve as part of the circuit. The system operates in one of two configurations: 1) with tissue construct discs interposed between the Windkessel and the reservoir or 2) with a valve-like construct in the position normally occupied by the VAD outflow valve. The system was water-filled and subjected to a hydrodynamic evaluation using high frequency response pressure transducers and an electro-magnetic flow probe downstream of the flow resistor. Analogue, pressure and flow signals recorded at a sampling frequency of 100 Hz were stored and analysed using proprietary hardware/software.

Data analysis demonstrated that the system was free from undesirable hydrodynamic characteristics and operated reproducibly over a wide range of conditions. Pulse pressure correlated with pump stroke volume and was inversely related to Windkessel air volume. Baseline Windkessel pressure was determined by flow and resistance. Thus, baseline and pulse pressure could be independently adjusted.

In conclusion, this system appears well-suited to the conditioning of tissue constructs to produce viable de-novo heart valve tissue.

(222) Hydroxyapatite/Collagen Composite Matrices for Bone Reconstruction

Ciardelli G., Gentile P., Mandrile R., Chiono V., Barbani N., Mattioli-Belmonte M., Vozzi G., Giusti P.

Politecnico in Turin, Department of Mechanics, Turin, C.so Duca degli Abruzzi, 24 I-10129, Italy

Bone tissue engineering is a promising approach for bone repair, because it may provide solutions for generating a new bone tissue with good functional and mechanical properties, reducing the risks and expenses of using autografts and allografts.

Three-dimensional porous hydroxyapatite/collagen (HA/CO) composites with a random pore structure were fabricated by freeze-drying, and cross-linked by an enzymatic treatment using transglutaminase (TGase) to improve physical characteristics such as mechanical strength and thermal stability. The scaffolds were characterized in terms of their stability (CO release, swelling, collagenase mediated degradation), chemical (infrared spectroscopy) and thermal properties (thermogravimetric analysis, differential scanning calorimetry), mechanical behavior under compression and cell compatibility (1).

Enzymatic treatment stabilized the sponges to water vapours, with measurable swelling ratio between 100% (for pure CO) to 5% for CO/HA 20/80. Weight loss in water due to CO release was between 2 and 10% in TGase cross-linked samples and decreased with increasing HA content.

Cultures of osteoblast cell lines MG 63 and human umbilical vein endothelial cells (HUVEC) showed good adhesion and proliferation on the scaffolds, good viability (through MTT test, 100-150% of control) and good differentiation (Alkaline phosphatase, up to 12 UI/l with respect to 8.6 UI/l for control).

Further improvement of the biomimetic properties of the scaffolds can be achieved with the incorporation of polymeric systems able to promote a light-controlled biomineralization (photodynamic biomineralization).

Reference

1. S. Yunoki, T. Ikoma, A. Monkawa, K. Ohta, M. Kikuchi, S. Sotome, K. Shinomiya and J. Tanaka. (2006). Control of pore structure and mechanical property in hydroxyapatite/collagen composite using unidirectional ice growth. *Material Letters*. 60: 999-1002.

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(223) Ibuprofen and Hydrogel Released Ibuprofen in the Reduction of Inflammation Induced Migration in Melanoma Cells

MacNeil S., Redpath M., Waddon A., Lalla R., Marques C.M.G.

Department of Engineering Materials, Kroto Research Institute, North Campus, University of Sheffield, Broad Lane, Sheffield S3 7HQ, United Kingdom

The involvement of inflammation in exacerbating cancer metastasis is under investigation with several clinical studies showing that

patients taking non steroidal anti-inflammatory drugs (NSAIDs) may have reduced metastases. The aims of this study were a) to examine the effects of Ibuprofen on the major pro-inflammatory cytokine TNF- α in inducing migration of C8161 and HBL human melanoma cell lines and b) to develop Ibuprofen releasing hydrogels for future topical use in reducing metastatic spread of primary melanoma. We found 300 U/ml TNF- α maximally stimulated migration of both C8161 and HBL melanoma cells in scratch assays over 24 h. To investigate the effects of Ibuprofen on TNF- α stimulated migration, cells were exposed to 300 U/ml TNF- α for a 24-h period prior to scratching and the addition of Ibuprofen. Unstimulated cell migration was not significantly affected by Ibuprofen up to 10^{-3} M for either cell type but Ibuprofen at 10^{-3} M significantly reduced TNF- α stimulated migration to that of non-stimulated cells ($p < 0.001$). No difference was found in the response of cells to SS and SR forms of Ibuprofen. The effect of an Ibuprofen sodium salt loaded hydrogel (Pluronic F127) was then examined on unstimulated and TNF- α stimulated C8161 cell migration. The unloaded hydrogel significantly reduced cell migration, however cells treated with 0.20 and 0.15% Ibuprofen loaded hydrogels showed a further significant reduction in migration when compared to unloaded hydrogel. In summary TNF- α up-regulates malignant melanoma migration *in vitro* and this can be reduced by Ibuprofen both in solution and delivered from a hydrogel.

(224) Identification of Minimal *In Vitro* Culture Parameters Necessary for the Proliferation and Phenotype Maintenance of Human Mesenchymal Stem Cells

Bryan N., Hunt J.A., Rhodes N.P.

University of Liverpool, Department of Clinical Engineering, Ground Floor, Duncan Building, Daulby Street, Liverpool, L69 3GA, United Kingdom

Introduction: Mesenchymal stem cells provide a means of overcoming the multitude of boundaries associated with embryonic stem cells as a multipotent cell source for applications. This research aimed to overcome some of the key obstacles by the derivation *de novo* of chemically defined, animal product free *in vitro* culture parameters necessary for the maintenance and differentiation of mesenchymal stem cells post isolation.

Methods: Media was synthesized completely *de novo* using defined concentrations of a minimal number of basal component inorganic salts. A combination of energy sources, amino acids and vitamins were added; with serum added up to 5%. Conformation of cell phenotype was evaluated using flow cytometry for the expression of a widely acknowledged putative mesenchymal stem cell surface marker subset; +CD29, CD34, +CD44, -CD45, +CD90, +CD105. Cell phenotype analysis was carried out using immunohistochemical staining for cell specific proteins and visualized by laser scanning confocal microscopy.

Results/Discussion: Serum could be replaced for a limited number of passages using combinations of proteins, steroids and growth factors, driving cells to confluence in similar time periods to the serum containing media. Novel minimal media was capable of maintaining cellular proliferation rates and phenotypes of several cell lines including mesenchymal stem cells for extended periods

in culture with additions of serum upto 5% (50 passages thus far for human fibroblast cells). Replacement of serum was conducted using combinations of elements elucidated *de novo* allowing cells to grow without the aid of any undefined adduct for a limited number of passages.

(225) Impacts of Vascular Endothelial Growth Factor and von Willebrand Factor in Tissue-Engineered Arterial Patch with Biodegradable Scaffold

Ichihara Y., Shin'oka T., Saito S., Matsumura G., Miyamoto S., Hobo K., Ikada Y., Kurosawa H.

Department of Cardiovascular Surgery, Tokyo Women's Medical University, Tokyo, Japan

Objectives: We previously demonstrated the feasibility of tissue-engineered vascular autografts (TEVA) and successfully applied TEVA in patients. The indication of TEVA, however, was limited to using under low-pressure systems, therefore, we developed a new biodegradable polymer which can be use for arterial grafts. The purpose of this study was to examine dynamic changes of endothelial markers, vascular endothelial growth factor (VEGF) and von Willebrand factor (vWF), in this polymer using RT-PCR.

Methods: We implanted newly developed biodegradable polymer, which composed of a poly-L-lactide acid (PLLA) woven fabric sheet and a copolymer of epsilon-caprolactone and lactide acid (P(CL/LA)), as Tissue-Engineered Arterial Patches (TEAP) into canine descending aorta with 1, 3 and 6 months ($n=4$) follow. Explanted TEAP were evaluated by histology and the expressions of VEGF and vWF mRNA in the TEAP were determined using semi-quantitative RT-PCR.

Results: After 1 month postoperatively, vWF positive endothelial cells were visible on the inner layer of TEAP in histological evaluation, and after 3–6 months, these cells accumulated on the luminal surface more clearly. The expressions of vWF mRNA at 1 month were higher than that of tissue at 3 months ($p < 0.05$), and then gradually increased to the level of native tissue at 6 months. The expressions of VEGF mRNA in the TEAP at 1 month were significantly higher than that of native arterial tissue ($p < 0.05$), and then decreased to the native level at 6 months.

Conclusions: These results suggest the maturation of endothelial cells in the TEAP. This novel biodegradable scaffold which enhances endothelialization can be a realistic material under high-pressure systems.

(226) Implantation of Porcine Cornea Decellularized by Ultra High Hydrostatical Pressurization to Rabbit Cornea

Kobayashi H., Kimura T., Funamoto S., Hashimoto H., Sasaki S., Mochizuki M., Fujisato T., Kishida A.

Biomaterials Center, National Institute for Materials Science, Tsukuba, Ibaraki, 305-0044, Japan
Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo, 11-0062, Japan

Department of Ophthalmology, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, 113-8510, Japan
 Department of Biomedical Engineering, Osaka Institute of Technology, Asahi-ku, Osaka, 535-8585, Japan

We have developed the porcine cornea decellularized by ultra high hydrostatical pressurization (UHP method), in which the cells were disrupted by ultra high pressurization and removed by washing process, to be utilized as novel artificial bio-cornea. We previously reported that the decellularization of porcine cornea was achieved by UHP method. In the present study, we investigated the implantation of porcine cornea decellularized by UHP method to rabbit cornea. Porcine corneal specimen with the thickness of 200 micro-meters was prepared using microkeratome. The specimen was pressed at 10,000 atm and 10 degree for 10 min, and then washed with cell culture medium containing 3.5% dextran for 72 hours. The semi-transparent cornea was obtained. By H-E staining, the complete removal of cells and the maintenance of the superstructure of collagen fibrils were confirmed. The porcine corneal discs having the diameter of 2 mm with/without the decellularization by the UHP method were implanted in a rabbit corneal pouch. It was found that the severe inflammation and vascularization occurred in the case of the native porcine cornea, which became opaque, in initial stage, whereas they were not observed for the implantation of the porcine acellular cornea, which became transparent. From these results, it suggests that the porcine cornea decellularized by the UHP method could be useful as an artificial corneal stroma for tissue regeneration.

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(227) Improved Adhesion of Osteoblast-Like Cells on Chitosan Membranes Surface Modified via Introduction of Phosphonic Groups

López-Pérez P.M.^{1,2}, Da Silva R.M.P.^{1,2}, Pashkuleva I.^{1,2}, *Reis R.L.^{1,2}

¹3B's Research Group—Biomaterials, Biodegradables and Biomimetics, Dept. of Polymer Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

²IBB—Institute for Biotechnology and Bioengineering, PT Government Associated Laboratory, Braga, Portugal

Chitosan has been proposed for a number of biomedical applications. Unfortunately, poor cell adhesion and proliferation on native chitosan membranes is an important limitation on its use.

This study proposes a method for improvement of osteoblast-like cells adhesion/proliferation via anionic moieties introduction on the surface of chitosan membranes. Vinyl phosphonic acid (VPA) was chosen as monomer because phosphates are expected to interact favourably with the mineral component of hard tissues and the polymers of VPA are non-toxic. Two-step modification procedure was used: (i) activation by Oxygen plasma treatment, followed by (ii) vinyl phosphonic acid graft polymerization.

The successful grafting was confirmed by X-ray photoelectron spectroscopy (XPS). New peak for phosphorus (133.2 eV) appears in the spectra of the modified membrane. Slightly high roughness

was measured (interferometry, optical profiler) for the treated samples most probably because of the etching processes ongoing during the plasma treatment.

Direct contact tests were performed using a human osteosarcoma cell line (SaOs-2). Cell morphology (optical and scanning electron microscopy) and cell viability (MTS test) were evaluated for untreated and surface modified membranes. The results revealed that the presence of phosphonic groups on the surface improves both SaOs-2 adhesion and proliferation when compared to untreated membranes. Optical density measurements indicate higher number of viable cells adhered to VPA modified surface compared to untreated material. Moreover, the cell morphology was also positively affected by the treatment.

Surfaces containing anionic moieties can be used on orthopaedic tissue engineering area, not only because of their biomineralization ability, but also because they stimulate adhesion and proliferation of bone-related cells.

(228) Improved Cardiac Function by Bio-Engineered Skeletal Myoblasts in MI Rats

Lee J.E.¹, Ju B.M.¹, Lim H.B.¹, Kim E.J.¹, Kim J.S.²

¹Cell Therapy Research Institute, BHK Inc., Seoul, 135-832, Korea

²College of Pharmacy, Sookmyung Women's University, Seoul, 140-732, Korea

Cellular cardiomyoplasty represents a novel therapeutic modality for the treatment of end-stage heart failure and the implantation of skeletal myoblasts has proved effective for the improvement of myocardial function. The engraftment rate of injected cells is, however, only around 10 % [1] so that the use of biomaterials has evolved to improve it [2]. In this study, various kinds of biomaterials were used to formulate the skeletal myoblasts to increase the bioretention of skeletal myoblasts in infarcted area, resulting in an improved fractional shortening (FS) and left ventricular ejection fraction (LVEF) in myocardial infarction (MI) rats. The histology and immunohistochemistry were also investigated. Liposomal formulation of myoblasts showed 24% and 27% increase in FS and LVEF, respectively, compared to control MI group as determined by echocardiography 5 weeks after injection. Pluronic formulation of myoblasts also showed 13% and 10% increase in FS and LVEF, compared to control MI group. Our results indicate that liposomal or polymeric formulation may serve as a good injectable internal support or scaffold for myocardial cell transplantation, delivering more viable cells directly into infarcted myocardium with higher retention rate. Further studies of cell adhesion, cell viability, and the efficacy of myoblasts in biomaterial formulations using other biomaterials are currently under going both *in vitro* and *in vivo*.

References

1. P. Zammaretti and M. Jaconi. (2004). Cardiac tissue engineering: regeneration of the wounded heart, *Current Opinion in Biotechnology* 15:430–434.
2. M.E. Davis, P. Hsieh, A.J. Grodzinsky and R.T. Lee. (2005). Custom design of the cardiac microenvironment with biomaterials, *Circulation Research* 97:8–15.

(229) Improved *Ex Vivo* Propagation of Endothelial Progenitor Cells by CYR61/CCN1

Wagemanns R., Hofmann A., Eck M., Schmauß B., Jatzke S., Nöth U., Jakob F., Schütze N.

University of Würzburg, Orthopedic Institute, Orthopedic Center for Musculoskeletal Research, Molecular Orthopedics, Würzburg, Germany

Endothelial progenitor cells (EPCs) have high potential in different applications such as vascularisation of tissue engineered grafts and treatment of ischemic regions. A major limitation until today is the small number of EPCs which can be obtained from one patient. Here we analysed the effect of the cysteine-rich protein 61 (CYR61/CCN1) on the propagation of endothelial progenitor cells.

EPCs obtained from peripheral blood (27 volunteers) via Ficoll gradient centrifugation were treated with 0.5 µg/ml CYR61 or left untreated (control). EPC marker expression of CD133, CD34 and KDR was examined using FACS-analysis, immunohistochemistry and RT-PCR respectively. Uptake of acetylated LDL (acLDL) and concurrent staining for ulex lectin was analysed as well. Dose dependency of CYR61 (range 0.05 – 1.5 µg/ml) effect on EPCs was investigated. Number of cultivated cells was determined after HE-staining.

Treatment with 0.5 µg/ml CYR61 leads to a 7-fold increased cell number of *in vitro* cultivated EPCs. Marker expression of CD133, CD34 and KDR was determined positive for CYR61 treated and untreated control cells. Double staining for both acLDL and ulex lectin occurred in the majority of cases in +/- CYR61 treated cells. EPCs responded to CYR61 treatment with enhanced cell number already in a low concentration of 0.05 µg/ml. From our data we conclude that CYR61 enhances adherence and endothelial differentiation of mononuclear cells and in addition may have a moderate effect on endothelial precursor proliferation. In conclusion the angiogenic inducer CYR61 is a powerful tool for *ex vivo* propagation of EPCs and further applications in tissue engineering.

(230) Improved Technologies for Translating Culture of Human Melanocytes to the Clinic

Eves P.¹, Bullett N.², Haddow D.², Gawkrödger D.³, MacNeil S.¹

¹The Kroto Research Institute, University of Sheffield, S3 7HQ, United Kingdom

²CellTran Limited, The Innovation Centre, Sheffield, S1 4DP, United Kingdom

³The Royal Hallamshire Hospital, Sheffield, S10 2JF, United Kingdom

While laboratory expanded autologous melanocytes have clearly been shown to be of benefit in the surgical treatment of patients with vitiligo, there is a need for a robust delivery and grafting strategy for patients. In this paper we present a convenient methodology for delivering cultured autologous melanocytes and keratinocytes from the laboratory to the patient which is low risk for the patient but also user friendly for the surgeon.

A chemically defined substrate (acrylic acid deposited by plasma polymerisation onto medical grade silicone backing dressing)

was used to culture both melanocytes and keratinocytes in medium (Greens) currently used in the clinic (which contains fetal calf serum sourced from New Zealand), but also in a serum free alternative—M2. We demonstrate successful transfer of melanocyte:keratinocyte co-cultures from these carriers onto an *in vitro* human wound bed model. The transferred melanocytes were capable of achieving pigmentation and the keratinocytes rapidly provided an epithelial barrier layer.

In conclusion we have developed a pre-clinical methodology for the delivery of co-cultures of autologous melanocytes and keratinocytes using a flexible and user-friendly chemically defined carrier surface. This should facilitate translation of this work from the laboratory to the clinic to benefit those patients with stable vitiligo who could be candidates for surgical treatment.

(231) *In Vitro* Analysis of the Inflammatory Potential of a Prototype Biomaterial Scaffold

Edwards F.C., Di Y., Dye J.F.

RAFT Institute, The Royal Free Hospital NHS Trust, Mount Vernon Hospital, Northwood, Middlesex, HA6 2RN, United Kingdom

A prototype fibrin-based, glutaraldehyde cross-linked composite scaffold developed in our laboratory. Using *in vitro* cell biological assays, the prototype showed no cytotoxicity and rapid endothelial cell ingress over 48 hr. However, evaluation in a porcine full thickness model revealed a significant acute inflammatory response to the material which compromised its scaffolding effectiveness. We have investigated a suitable *in vitro* assay to identify the source of this response.

Human neutrophils (Nø) were aliquoted with test samples for 1 hr before immunostaining for activated CD18 and flow cytometry. PBS/glucose or hepes/saline/glucose were used as a unreactive Nø assay buffers and isolation conditions were optimised to minimise spontaneous activation. 5 µM PMA was used as a standard control stimulant. Scaffold components (fibrinogen, fibrinogen degradation product, glutaraldehyde, alginates, calcium chloride) were tested over the potential concentration ranges released by scaffold degradation. Cytotoxic effects (on acute viability and proliferation) were assayed with human dermal fibroblasts by MTS reduction.

Neither fibrinogen, fibrinogen degradation fragments, nor alginates elicited significant Nø activation, nor fibroblast cytotoxicity. Nø activation by GTA release from scaffolds was prevented by chemical reduction. However, Nø were potently activated by Ca²⁺, with significant activation at 5 µM and maximal 10 µM, with similarly potent cytotoxic effects on fibroblasts.

Apart from GTA, the identification of Ca²⁺ as a potent mediator of foreign-body inflammation and cytotoxicity will guide future development of the proposed scaffold. With corroboration by other assays of Nø activation (elastase, TNF), the method promises to be a useful addition to the tissue engineer's toolkit.

(232) *In vitro* Assessment of the Cytotoxicity and Inflammatory Potential of Glutaraldehyde as a Crosslinking Agent for Protein Scaffolds

Edwards F.C., Di Y., Dye J.F.

RAFT Institute, The Royal Free Hospital NHS Trust, Mount Vernon Hospital, Northwood, Middlesex, HA6 2RN, United Kingdom

Prototype fibrin-based scaffolds crosslinked with glutaraldehyde (GTA) are under development and evaluation as a synthetic dermal replacement in our laboratory. Not only must the use of GTA be carefully controlled to optimise crosslinking, also the product must ultimately be assessed for safety and biocompatibility. Although GTA use in tissue engineering is well-established, it causes problems (e.g. foreign body reactions, calcification). Despite this, the mechanisms of toxicity are poorly understood.

Fibrin scaffolds were GTA crosslinked, NaBH₄-reduced and lyophilised. Supernatants were collected from samples soaked in PBS/glucose. Crosslinked fibrinogen films were digested in 6 M HCl to yield a Schiff-base hydrolysate, which was dried, dissolved in PBS/G and neutralised. GTA standards were also prepared in PBS/G. Cytotoxicity from exposure of human dermal fibroblasts to test samples was examined using short-term viability and 3 day proliferation. Inflammatory potential of test samples was assessed by the activation of human neutrophils (Nø) exposed to samples for 1 hr.

GTA LD₅₀ was 50–150 µM (viability) and 15–50 µM (proliferation) broadly corroborating literature results. Non-reduced but not reduced matrix supernatants gave partial cytotoxicity (15–50 µM GTA-equivalent). A novel finding was that GTA stimulates profound Nø activation (ED₅₀ 50–150 µM) and non-reduced but not reduced matrix supernatants elicited Nø activation (15–50 µM GTA-equivalent). The GTA release profile from non-reduced matrices correlates with these data. Another new finding, that Schiff-base products cause cytotoxicity and Nø activation, identifies a further mechanism for adverse reactions to GTA-crosslinked materials.

Together this extends our understanding of GTA toxicity and suggests achievable limits for Schiff-base adducts and released GTA.

(233) *In Vitro* Fabrication of Tissue-Engineered Bones and *In Vivo* Transplantation

Song K.D., Liu T.Q.

Dalian University of Technology, Dalian R&D Center for Stem cell & Tissue Engineering, Dalian 116024, P.R. China

In vitro fabrication of tissue-engineered bones with the BDBS (bio-derived bone scaffolds) in RWVB (rotating wall vessel bioreactor) and transplantation by using these constructs to repair the segmental bone defects of Zelanian rabbit were studied. In this paper, the osteoblasts, after being transfected with green fluorescent protein (GFP), were seeded at 1×10^6 cells/ml onto the bio-derived bone scaffolds and then cultured in RWVB for one week. For a comparative study, cell seeded constructs were also cultured in static conditions. The morphologies and structure of the fabricated bones were investigated by inverted microscope, SEM (scanning electron microscope), and stains of HE (haematoxylin-eosin) and toluidine blue. Then, the animal experiment in repairing segmental bone defects of Zelanian rabbit by using the above tissue-engineered bone fabricated in RWVB was operated. The results show that the cell number cultured in RWVB was five times as that in T-flask. These tissue-engineered bones cultured in RWVB grew well. The bone defects were almost repaired in the experimental group 8 weeks after the implantation, showing the best results

among the 3 groups. It demonstrates that with the stress stimulation in RWVB, the ALP expression can be increased, a rapid proliferation and differentiation of osteoblasts are possible, and the 3D fabrication of tissue-engineered bones could be realized. The tissue-engineered bone constructs fabricated in RWVB possess good ability in repairing segmental bone defect.

(234) *In Vitro* Study of Biotolerance of Cycloolefin Copolymer/Polyethylene Blends

Hulejová H.¹, Šenolt L.¹, Polanská M.¹, Petrtýl M.², Bastl Z.³, Kruliš Z.⁴, Horák Z.⁴

¹Institute of Rheumatology, Prague, CZ

²Czech Technical University, Faculty of Civil Engineering in Prague, CZ

³Institute of Physical Chemistry, Academy of Sciences, Prague, CZ

⁴Institute of Macromolecular Chemistry, Academy of Sciences in Prague, CZ

Introduction: Development and application of new materials for artificial substitutions of skeletal elements is addressed to materials which have similar mechanical features to biomechanical characteristics of organic tissues and are tolerated by biological milieu. These requirements are fulfilled by the cycloolefin copolymer/polyethylene (COC-blend). The modification of selected mechanical properties of COC via blending with polyolefinic elastomers and/or reinforcing with carbon fibers potentially proposed for various artificial parts of prostheses (cartilages, damping of replacement elements etc.). In our study we monitored the biotolerance of this material in the culture of chondrocytes *in vitro*.

Material and Methods: Two types of materials (COC and COC-blend) either coated with collagen type II or uncoated were tested *in vitro*. For better adhesion of collagen, the surface was modified with N and O ions. Spontaneous proliferation of chondrocytes was investigated by establishing of growth curves. The cell adhesion was tested by labeling cells with 5'-bromo-2'-deoxy-uridine (BrdU). Apoptosis (active caspase-3), production of some inflammatory cytokines and metalloproteinases were measured in medium or cells by ELISA and by the method of molecular biology.

Result and Discussion: Both materials COC as well as COC-blend showed similar biological properties with respect to proliferation and apoptosis of chondrocytes. Expression of several extracellular matrix-degrading enzymes and pro-inflammatory cytokines in chondrocytes were similarly regulated by these two materials. Taken together, it can be suggested that COC as well as COC-blend can represent suitable materials for artificial substitution of osteochondral defects.

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(235) *In Vivo* Development and Long-Term Survival of Engineered Adipose Tissue Depend on *In Vitro* Precultivation Strategy

Weiser B.¹, Prantl L.², Schubert T.³, Zellner J.², Fischbach-Teschl C.⁴, Spruß T.¹, Seitz A.¹, Tessmar J.¹, Göpferich A.¹, Blunk T.¹

¹Department of Pharmaceutical Technology, University of Regensburg, Germany

²Institute of Trauma and Reconstructive Surgery, University of Regensburg, Germany

³Institute of Pathology, University of Regensburg, Germany

⁴Department of Biomedical Engineering, Cornell University, Ithaca, NY, United States of America

One strategy of adipose tissue engineering is to transplant adipocytes or adipogenic precursors in combination with polymeric materials. However, satisfying formation of fat tissue and long-term survival still remain major problems. There is increasing evidence that treatment of the cells prior to implantation plays a critical role in the success of adipose tissue growth. In a previous study we established a model system based on 3T3-L1 cells that allows reproducible engineering of mature, coherent adipose tissues *in vitro* [1]. Utilizing this model system the current study now investigated systematically the long-term *in vivo* development of cellular constructs with varying stages of adipogenic development at the time point of implantation.

Therefore blank PGA fiber meshes, scaffolds seeded with uninduced 3T3-L1 preadipocytes as well as seeded cell-polymer constructs precultivated under adipogenic conditions for 2, 9 or 35 days were implanted subcutaneously into nude mice. Histological analysis revealed that no fat formation occurred in constructs without adipogenic precultivation. Implantation of mature fat pads (35 d of adipogenic precultivation) resulted in adiponecrosis within the constructs. In contrast, constructs adipogenically precultivated for 2 or 9 days with an immature phenotype at the time point of implantation gave subsequently rise to vascularized, mature adipose tissue *in vivo* (80% fat area, determined by histomorphometry). Furthermore, these engineered adipose tissues showed long-term survival *in vivo* over the whole investigation period of 24 weeks. The gained insights can now contribute to the development of a clinical setup for soft tissue reconstruction.

Reference

1. Fischbach et al. (2004) Exp. Cell Res. 300. pp. 54–64.

(236) *In Vivo* Functionality of Goat Marrow Cells Seeded onto SPCL Scaffolds Constructs in Induced Non Critical Femoral Defects

Rodrigues M.T.^{1,2}, Gomes M.E.^{1,2}, Viegas C.A.^{1,2,3,4}, Azevedo J.T.^{3,4}, Dias I.R.^{1,2,3,4}, Reis R.L.^{1,2}

¹3B's Research Group—Biomaterials, Biodegradables and Biomimetics, Dept. of Polymer Engineering, University of Minho, Braga, Portugal

²IBB—Institute for Biotechnology and Bioengineering, PT Government Associated Laboratory, Braga, Portugal

³Dept of Veterinary Sciences, Univ. de Trás-os-Montes e Alto Douro, Quinta dos Prados, 5000-911 Vila Real, Portugal

⁴Dept of Animal Science, Univ. de Trás-os-Montes e Alto Douro, Quinta dos Prados, 5000-911 Vila Real, Portugal

Functional bone regeneration strategies require cells with high proliferation and osteogenic potential, as marrow stromal cells, and a suitable scaffold to support cell development towards new bone tissue formation.

This study aims to assess the *in vivo* osteogenic functionality of constructs based on goat marrow stromal cells (GBMCs) and starch-policaprolactone (SPCL) scaffolds cultured *in vitro* at different stages of development using an autologous approach.

GBMCs harvested from iliac crests of adult goats were cultured with autologous serum before *in vitro* seeding onto SPCL scaffolds (d = 6 mm/h = 2 mm). Afterwards, cells were cultured in osteogenic medium containing dexamethasone, ascorbic acid and B-glycerophosphate for 1 or 7 days prior to implantation. An *in vitro* control was considered to assess cell proliferation and differentiation by DNA quantification and ALP activity, respectively. Non-critical defects (d = 6 mm/3 mm depth) were drilled in two femurs of four goats: in each femur, two drills were left empty, two were filled with SPCL alone (controls), and the remaining were filled with GBMCs seeded onto SPCL for 1 or 7 days, respectively. After implantation, intravital fluorescence markers (xylenol orange, calcein green and tetracycline) were injected subcutaneously after 2, 4 and 6 weeks, respectively, to assess the different stages of new bone formation. Six weeks after implantation animals were euthanized, the femurs cut into single defect-sections and observed for fluorescence detection or stained with Levai LacZKO.

No significant inflammatory response to the implanted constructs was observed due to an excellent regeneration process. New bone was observed in all defects, but these observations also suggest a higher bone growth in cell-SPCL constructs defects when compare to empty or SPCL alone defects. This may suggest that bone growth is enhanced by the presence of cell-scaffold constructs and that the *in vivo* culturing time seems to play an important role in bone growth onto these defects.

(237) *In Vivo* Generation of Functional Tissues Using the Inkjet Printing Technology

Xu T., Zhao W., Atala A., Yoo J.

Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, United States of America

Introduction: Organ printing using the inkjet technology has evolved into a promising approach for engineering new tissues or organs. In this study we investigated whether the printed multi-cell derived tissue constructs could maintain their structural and spatial orientation *in vivo*. We examined whether these tissues are able to survive and mature into functional tissues when implanted *in vivo*.

Materials and Methods: Three-dimensional multi-cell constructs with a “pie” configuration were fabricated by simultaneously printing 3 different cell types (muscle, endothelial and human amniotic fluid stem cells) into collagen/alginate gel. The cells were labeled with 3 different membrane bound tracers, printed and implanted subcutaneously into athymic mice. The implanted constructs were monitored by micro-CT scanner over time (up to 18 weeks) and retrieved for analyses.

Results and Discussion: A complete 3D “pie” shaped construct containing 3 different cell types was successfully fabricated. The tissue structure of the 3D tissue was maintained and the printed cells remained viable in their designated locations. Formation of each tissue components with their respective function (bone, muscle and vessel) was confirmed by confocal, immunohistochemistry and Micro CT scanning.

Conclusions: This study shows that multi-cellular constructs generated by the inkjet method are able to maintain their structural and spatial orientation *in vivo*. The inkjet printing technology may become a standard method of engineering functional tissues for clinical applications.

(238) Induction of Endothelial Differentiation of Adult Rat Mesenchymal Stem Cells in a Collagen Glycosaminoglycan Scaffold

Farrell E., O'Brien F.J.,

Royal College of Surgeons in Ireland and Trinity College Dublin, Ireland

One cause of failure of implanted tissue engineered constructs is lack of nutrient delivery and waste removal from the construct centre. Core degradation and cellular necrosis at the centre of tissue engineered scaffolds *in vivo* is a major problem due to lack of vascularisation. This is caused by insufficient time for blood vessel infiltration from the surrounding tissue. The aim of this research was to induce angiogenesis from marrow stromal cells in a collagen glycosaminoglycan scaffold as a first step to developing a vascularised tissue engineered bone construct. Collagen glycosaminoglycan scaffolds were seeded with adult mesenchymal stem cells derived from Wistar rats for up to 3 weeks prior to treatment. Cell-scaffold constructs were then treated with 50 ng/ml VEGF for up to three weeks in either medium containing 10% (high) serum or 1% (low) serum containing medium. Following this period, scaffolds were retrieved and analysed immunohistochemically for von Willebrand factor using laser scanning confocal microscopy. Results from this preliminary study showed that endothelial differentiation of adult rat MSCs was possible. Furthermore culture of cells in low serum alone proved to be sufficient for cells to express von Willebrand factor. It was observed that a combination of high serum culture for 3 weeks followed by low serum culture in the presence of VEGF for 3 weeks gave optimum results with regard to both cell number and von Willebrand factor expression. The next steps in this work will aim to optimise neovascularisation in these scaffolds using a combination of growth factors mechanical stimulation and a hypoxic environment.

(239) Induction of Shape Memory and 3D Structure Into a Biodegradable Collagen Membrane for Tissue Engineering

Foroughi F.¹, Behjati B.², Brown R.A.¹

¹University College London, Tissue Repair and Engineering Centre, Institute of Orthopaedics, London, HA7 4LP, United Kingdom

²MBP, Neustadt-Glewe, Germany

Standard insoluble collagen (haemostatic) sponges are in wide clinical use and degrade biologically over approximately 30 days. When such foam scaffolds (MBP, biomaterials, Germany) are air dried (rather than freeze dried) they give a dense, non-porous, translucent film (<0.5 mm). Present attempts to fabricate urothelial implants require strong, biodegradable scaffolds (preferably collagen/biomimetic) in the form of tubes/domes, which are able to support 3D cell growth. We investigated here the response of

neonatal human dermal fibroblasts (HDF) to this potential cell scaffold and the ability to generate controlled 3D structure. Membranes were partially swollen/remodelled by soaking for either 24 or 48 h in 0.2 M acetic acid, after which they were neutralised by washing in PBS. Ideal swelling time was 48 h, and produced membrane surface roughening by raising 'fingers' of collagen (range 10–70 µm diameter approximately). Secondly, acid swollen membranes could be formed into any given shape (e.g. cylinder) which was retained rigidly after neutralising. This shape-memory effect and surface structuring were characterised by scanning electron microscopy. Cell utilisation of the pseudo-3D substrate was excellent with around 90% cell viability (10⁴ cell seeding) after 72 h with growth deep into and between the surface fingers. It is concluded that the treatment of clinically available collagen membranes represents a simple achievable source of the basic cell supports and tissue structure needed for urothelial engineering.

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(240) Influence of Angiogenesis by New Synthetic Peptides and Peptidomimetics

Horváth A.¹, Seprődi J.¹, Vántus T.¹, Bökönyi G.¹, Tanai H.¹, Tóvári J.², Kenessey I.², Kéri G.¹

¹Peptidbiochemistry Research Group of the Hungarian Academy of Sciences and Semmelweis University, Department of Medicinal Chemistry, Cooperative Research Centre, Budapest, Hungary
²Department of Tumor Progression, National Institute of Oncology, Budapest, Hungary

We targeted VEGF and HIF in our strategy to find new angiogenesis influencing compounds.

1. Vascular endothelial growth factor (VEGF) family members, VEGF-A and VEGF-ENZ-7 (encoded in the ORF virus genom) have high affinity to VEGFR-2 at a similar level. These factors, in their 3D structures, form three-loops from which loops 1 and 3 are essential for biological activity (1). Based on the primary structure of these loops we designed and synthesized small peptides and tested their effect on angiogenesis *in vitro* and *in vivo*. VEGF-ENZ-7-fragment showed 3 fold new vessel formations at 10 mg/kg dose compared to control.

2. Hypoxia Inducible Factor (HIF) activates genes involved in angiogenesis (e.g. VEGF). It can be stabilized by inhibiting prolyl hydroxylase (PHD1) (2). Molecules carrying certain functions (e.g. small peptides) are able to influence PHD1 pathway through the iron coordination motif. We synthesized several small peptides and peptidomimetics and tested their effect on angiogenesis both *in vitro* and *in vivo*. One of our small peptide-derivative showed *in vivo* 10 fold new vessel formation compared to control and another compound inhibited endothelial cell proliferation by 95% at a dose of 5 µM.

References

1. Hewiston, et al. (2004). Drug. Disc. Today 9, 704–711.
2. A. Kiba, et al. (2003). J. Biol. Chem. 278, 13453–13461.

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(241) Influence of Different Collagen-Based Scaffolds (Sponge or Type I and II Gels) on the Phenotypic and Collagenases Expression of Bovine Chondrocytes

Freyria A.M., Ronzière M.C., Cortial D., Herbage D., Mallein-Gerin F.

Institut de Biologie et Chimie des Protéines (IBCP UMR 5086); CNRS; Univ. Lyon 1; IFR 128 BioSciences Lyon-Gerland; 69367 Lyon Cedex 7, France

The purpose of this study was to compare in systematic experiments the phenotype of calf articular chondrocytes and their gene expressions for collagenases (MMP-1 and -13) and collagen-binding receptors (integrin families and discoidin-domain receptor families) when cultured 12 days within different bovine collagen-based scaffolds.

Chondrocytes were seeded in collagen sponges (2×10^6 cells/ml) and in gels (2×10^6 cells/ml) made of acid soluble collagen type I (1.25 mg/ml), pepsin-treated type I and papain-treated type II (3 mg/ml). The gels were maintained in both attached and floating states under static conditions. The behaviour of the cells were followed regarding their proliferation and gene expressions (types I, II collagens, aggrecan, MMP-1, -13, $\alpha 1, \alpha 2, \alpha 10, \alpha 11$ -integrin subunits, DDR1 and DDR2) using real-time PCR.

After 12 days minimal contractions were observed for the sponges (15%) and for the attached Col1 gels, if compared to the contractions of the floating col1 and col2 gels (50-60%). The phenotype (measured by the ratio Col2A1/Col1A2 mRNA) was better maintained in contracted gels (ratio >70) than in attached gels (ratio <40). The lowest value (ratio = 3) was obtained in sponges seeded with 2×10^6 cells/ml. Large increases in MMP-1 (20 to 50-fold) and MMP-13 (100-fold) expression were observed for the cultures in gels whereas the levels of expression of DDR-1 and 2, similar at day 0 were not increased.

This study demonstrates that the chondrocyte behaviour in collagen matrices varies more with the physical characteristics of the scaffolds (sponge or gel, contraction or not) than with the collagen type (I or II) used to make them.

(242) Influence of Fiber Diameter in Electrospun Polycaprolactone Scaffolds for Functional Tissue Engineering

Balguid A., Van Marion M., Mol A., Bouten C., Baaijens F.

Eindhoven University of Technology, Department of Biomedical Engineering, Building W-hoog, PO Box 513, 5600 MB, Eindhoven, The Netherlands

Electrospinning has been used to fabricate scaffold meshes with fiber diameters ranging from 7 nm to 15 μ m. In terms of dimensions, fibrillar structures in the extracellular matrix (ECM) range in nanometer scales. The use of electrospun nanofiber scaffolds in tissue engineering (TE) appears promising to replicate the dimension of natural ECM. However, cell ingrowth is reported to be compromised in electrospun scaffolds.

The objective of this study was to investigate the relation between fiber diameter of electrospun poly- ϵ -caprolactone (PCL) scaffolds and cell ingrowth. Five groups of electrospun PCL scaffolds with increasing fiber diameter were seeded with human myofibroblasts and cell ingrowth was analyzed after three days of culture. Additionally,

tissue development was studied in the scaffolds after four weeks of culture. A scaffold of poly-glycolic acid coated with poly-4-hydroxybutyrate (PGA-P4HB) served as a reference material.

The results showed that cell ingrowth of human myofibroblasts increased proportionally with increasing fiber diameter of electrospun PCL scaffolds. This suggests that fiber thickness is a crucial parameter in the design of functional electrospun scaffolds for TE. From the four week experiment it was seen that glycosaminoglycan and collagen production was higher in PCL constructs compared with PGA-P4HB. These differences might be explained by the differences in degradation rate and/or degradation products of the scaffold materials.

In conclusion, electrospun PCL scaffolds are suitable for TE, provided that the fiber diameter is sufficiently large. It allows proper cell ingrowth and has a relatively large surface area, which is beneficial for cell attachment and ECM biosynthesis.

(243) Influence of Fibrin Gel-Immobilized Growth Factors on Angiogenesis in the Subcutaneous Implantation Model

Arkudas A., Horch R.E., Tjiawi J., Polykandriotis E., Beier J.P., Bleiziffer O., Kneser U.

University of Erlangen Medical Center, Department of Plastic and Hand Surgery, Krankenhausstrasse 12, 91054 Erlangen, Germany

Introduction: Our aim was to quantitatively assess the angiogenic effects of VEGF and bFGF immobilized in a fibrin-based drug delivery system in the subcutaneous rat model.

Methods: Four teflon isolation chambers containing fibrin gel matrices were implanted subcutaneously in an upside down fashion at the back of 30 Lewis rats. The matrices composed of 500 μ l fibrin gel with two different fibrinogen concentrations (10 mg/ml or 40 mg/ml fibrinogen) and 2 IU/ml thrombin, loaded with VEGF and bFGF in five different concentrations (0 to 250 ng/ml each). At 3, 7, and 14 days after implantation, 4 matrices per group and time point were explanted and subjected to histological (Lectin immunohistology) and morphometrical analysis.

Results: All animals tolerated the operations well. At 1 week volume of the fibrin clots was significantly smaller in the 100 and 250 ng/ml VEGF and bFGF groups in comparison to lower concentrated growth factors. At 1 and 2 weeks the use of growth factors in low concentrations (25 ng/ml VEGF and bFGF) significantly increased the amount of fibrovascular tissue, average fraction of blood vessels and number of blood vessels at the matrix-host interface in comparison to growth factor-free controls. Higher concentrations did not further increase tissue formation and sprouting of blood vessels in this model.

Conclusion: This study demonstrates that fibrin gel-immobilized angiogenic growth factors efficiently stimulate generation of fibrovascular tissue and sprouting of blood vessels in the subcutaneous model. The angiogenic effects display a saturation kinetic with an optimum between 25 and 100 ng/ml.

(244) Influence of the Extra Cellular Matrix on the Frictional Properties of Tissue Engineered Cartilage

Plainfosse M., Crawford A., Hatton P., Katta J., Jin Z., Fisher J.

Centre for Tissue Engineering and Biomaterials, University of Sheffield, S10 2TA, Sheffield, United Kingdom

Introduction: Articular cartilage provides effective bearing surfaces, able to sustain a remarkably low lubrication in diarthrodial joints for many decades. Its tribological properties may however be impaired following injury or disease and repair is made difficult by a relatively poor capacity for regeneration. Recognition of these problems has led to the development of tissue engineered (TE) cartilage which is potentially implantable for repair of cartilage lesion.

Objective: This study investigated the importance of the extra cellular matrix on the frictional properties of TE cartilage.

Methods: TE cartilage was produced using published methods (Crawford and Dickinson). Indentation, initial and dynamic friction tests were performed on engineered constructs in a bath of lubricant (PBS). Native articular cartilage was used as a reference. The presence of glycosaminoglycan, type I and II collagens and lubricin were investigated to characterise the constructs before and after mechanical testing.

Results: The engineered constructs exhibited a time-dependent increase during the friction tests. While this phenomenon was similar to native cartilage, the values were different. Evidence of tissue damage was observed for the engineered constructs. Biochemical and histological examinations showed similarities between engineered and native tissues but suggested that the engineered tissue was depleted in some components.

Conclusion: Engineered cartilage showed low frictional properties, but the phenomenon responsible for it was unclear. The debris of tissue released in the lubricant may have contributed to the low values recorded. The data presented suggest that advanced constructs with properties closer to those of native cartilage must be developed before clinical application.

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(245) Injectable Atelocollagen Type II Scaffold for Intervertebral Disc Regeneration

O'Halloran D.¹, Grad S.², Alini M.², Pandit A.¹

¹National Centre for Biomedical Engineering and Science, National University of Ireland, Galway, Ireland

²AO Foundation, Davos, Switzerland

In the area of intervertebral disc (IVD) tissue engineering, most of the research to date has targeted the inner nucleus pulposus (NP) of the disc. Our research lends itself to the development of an injectable in situ curable, atelocollagen type II based scaffold that can be used as a carrier for cells into the degenerated NP area post-nucleotomy. The behaviour of NP cells that were seeded within two varying atelocollagen type II scaffold concentrations (3 and 5 mg/ml) was investigated in this study. Rheological analysis showed that the scaffolds recovered their structural integrity after injection through a needle of 1mm diameter. Cells seeded in the 5 mg/ml scaffold had a higher DNA content ($6.65 \pm 0.22 \mu\text{g DNA}$)

compared to the 3 mg/ml scaffold ($5.04 \pm 0.15 \mu\text{g DNA}$). The 5 mg/ml scaffolds had $23.16 \pm 1.28 \mu\text{g sGAG/DNA}$ compared to $28.06 \pm 2.86 \mu\text{g sGAG/DNA}$ for the 3 mg/ml scaffolds. However, with respect to sGAG retention within the matrix, the 5 mg/ml scaffolds retained $10.81 \pm 0.48\%$ and the 3 mg/ml scaffolds retained $3.03 \pm 3.03\%$. Histological staining showed a more homogenous distribution of cells within the 5 mg/ml scaffold compared to the 3 mg/ml scaffold. Both cell seeded scaffold concentrations showed extensive volume reduction after 7 days in culture, with the 5 mg/ml scaffold displaying significantly less reduction compared to the 3 mg/ml scaffold. In conclusion, a composite atelocollagen type II based scaffold for NP regeneration has been optimised with 5 mg/ml concentration being the ideal concentration.

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(246) Inkjet Biofabrication: 3D Hydrogel Fabrication Containing Nano-Particles

Nakamura M.^{1,2}, Nishiyama Y.¹, Henmi C.¹, Yamaguchi K.^{1,2}, Mochizuki S.^{1,3}, Takiura K.^{1,4}, Nakagawa H.¹

¹Kanagawa Academy of Science and Technology, ²Tokyo Medical and Dental University, ³Osaka Institute of Technology, ⁴Yamagata University, Japan

One of the major issues in tissue engineering is to make thick and complicated tissues, which are composed of various types of cells and have micro-scaled structures and are sufficiently vascularized. Considering such characteristics, we have developed "Inkjet 3D biofabrication" as a technology to construct designed micro- to macro-structures of hydrogel with handling different multi-types of cells. In our technique, gel precursor is ejected into the substrate with gel reactant, and 3D hydrogel architectures can be constructed. Using cell suspended gel precursor, 3D hydrogel structures containing living cells can be made. And various materials can also be added into hydrogel, as well as living cells. Then, we tried to add nano-particles, because several functional nano-particles have been developed with nanotechnologies such as fluorescent nano-particles, protein coated nano-particles, drug delivery nano-particles, etc. On behalf of such functional nano-particles, the fluorescent latex nano-particles of 250 nm in diameter were tested in this study. Sodium alginate solution suspended with them was ejected into calcium chloride solution by inkjet. Micro gel beads, micro gel fibers and 3D hydrogel structures were successfully made containing nano-particles. We also confirmed that the special function of added nano-particles, fluorescence in this case, can also be added in fabricated 3D structures. Drop on demand inkjet 3D biofabrication technique using such functional nano-particles will contribute to the further developments in tissue manufacturing technologies.

(247) Innervation and Muscle Cell Infiltrates in Tissue-Engineered Collagen Constructs *In Vivo*

Jones S.¹, Morgan M.¹, Lewis M.², Brown R.A.¹, Mudera V.¹

¹Tissue Repair and Engineering Centre, University College London, Stanmore Campus, United Kingdom

²Division of Biomaterials and Tissue Engineering, Eastman Dental Institute, University College London, United Kingdom

Conventionally, tissue engineering aims to convert an initial cell-scaffold construct into a tissue-like architecture, with bio-mimetic function. This occurs through cell-mediated remodelling *in vitro* and has proved to be slow, costly and difficult to control. Using a novel cell-independent technique plastic compressed cellular and acellular constructs were fabricated (1). These constructs were implanted across inter-costal spaces in a rabbit model designed to provide cyclical tensile loading *in vivo* for up to 5 weeks. We have previously shown that cell seeded constructs elicited increased cellular infiltration, angiogenic response, mechanical integrity and decreased inflammatory response compared to acellular constructs (2). We have now tested these constructs for innervation using staining for Schwann cells (S100) and myoblast (CD56) infiltration.

Constructs were harvested (2), sectioned and stained for Schwann cells (S100) and myoblast (CD56) using immunocytochemistry and cell numbers quantified. Nuclei were counterstained with DAPI.

Pre-implantation constructs showed no CD56 +ve or S100 +ve cells. At 1 week, CD56 +ve cells were seen in all constructs. By 5 weeks, CD56 +ve were present throughout the cellular constructs compared to only at the periphery of the acellular group. S100 +ve cells were seen in all constructs by 1 week but were visualized as individual cells, but by 5 weeks in the cellular group, there was evidence of bundling of cells in the core compared to acellular constructs where isolated cells were only seen at the periphery.

References

1. Brown, R.A., Wiseman, M., Chuo, C.B., Cheema, U., & Nazhat, S.N. 2005, Advanced Functional Materials (15)11, 1762–1770.
2. Mudera, V., Morgan, M., Cheema, U., Nazhat, S. N., & Brown, R.A. Tissue Engineering and Regenerative Medicine 2007. Accepted for publication.

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(248) *In-Situ* Differentiation of Muscle Derived Cells Under a Mechanical Stimulus

Aggarwal S., Mudera V., Lewis M.P.

UCL, Department of Tissue Engineering, EDI & RNOH, United Kingdom

Introduction: Mammalian muscle possesses unique cell renewal property due to the presence of satellite cells, a population of tissue specific mesenchymal stem cells capable of multilineage differentiation (myogenic, osteogenic, chondrogenic, adipogenic) [1], a readily available source of stem cells. Although cell transplantation has been used for tissue repair, a biological scaffold is required for replacing tissue loss. Collagen as a scaffold is largely conserved across species and a major component of extracellular matrix. Its mechanical properties can now be improved by cell independent plastic compression (PC) with minimal loss of cell viability [2]. We now propose to evaluate the differentiation lineage of labelled

muscle derived cells embedded in PC collagen constructs under dynamic mechanical stimulation *in vivo* in a lapine model.

Methods: Allogenic cells from craniofacial (masseter) and thigh (rectus femoris) muscles were embedded in plastic compressed collagen constructs. These constructs were implanted up to 5 weeks into the intercostal spaces of female New Zealand rabbits. Constructs are evaluated post implantation using H&E, immunocytochemistry to test lineage, inflammatory response, host cell infiltration and remodelling and dynamic mechanical analysis for mechanical integrity.

Results: Pilot experiments to test feasibility have shown recovery of a viable construct at day 10 post-implantation. Gross analysis showed no host rejection to the construct, with an envelope of host tissue. Histologically there was a presence of a large number of infiltrating cells surrounding the construct and migrating along the layers of then rolled construct. Ongoing experiments will evaluate these constructs for mechanical integrity, maturation and lineage at later time points.

References

1. Sinanan A, Buxton PG and Lewis MP. Muscling in on Stem Cells. Biol. Cell 2006; 98: 203–214.
2. Brown RA, Wiseman M, Chuo C, Cheema U, Nazhat SN. Ultrapid engineering of Biomimetic Materials and Tissues: Fabrication of Nano- and Microstructures by Plastic Compression. Advanced Functional Materials 2005; 15:1762–1770.

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(249) Integrative Tissue Response and Prolonged *In Situ* Life of Minimally Crosslinked CEM

Burugapalli K., Chan J.C.Y., Kelly J.L., Pandit A.

National Centre for Biomedical Engineering Science, National University of Ireland, Galway, and Department of Plastic, Reconstructive and Hand Surgery, University College Hospital, Galway, Ireland

Intact extracellular matrices (ECM) available for clinical use either undergo rapid degradation or remain at the site of implantation long after their intended function. It is hypothesised that intact ECM matrices, with lower degree of crosslinking, would result in scaffolds with desired *in situ* degradation rate and reduced antigenicity. In this study, the *in situ* degradation and tissue response of minimally crosslinked cholecyst-derived extracellular matrix (CEM) were investigated.

CEM was crosslinked with 0.0005 and 0.0033 mM of EDC and NHS per mg CEM in 50 mM MES buffer (pH 5.5). Non-crosslinked CEM and GA tanned bovine pericardium (BP) (Peri-Strip[®], Biovascular Inc.) were the controls. 1×1.5 cm² CEM, CEMxED C0005, CEMxEDC0033 and BP samples were immobilised at subcutaneous level using a polypropylene suture in Sprague Dawley rats. The animals were sacrificed at 7, 28 and 63 days (*n* = 8 per scaffold per time point). Haemotoxylin and Eosin and Masson's trichrome stained sections were used for histology and stereological analysis.

All scaffolds were surrounded by a thin fibrous tissue rich in collagen and vasculature. CEM and CEMxEDC samples were infiltrated completely with host tissue and blood vasculature, while host tissue failed to infiltrate BP. The implant area decreased significantly from 7 to 63 days for CEM, while no significant differences were observed for the rest of the scaffolds. Acute inflammation was resolved within 7 days. The chronic inflammatory phase was predominated by macrophages. It is concluded that crosslinking significantly prolonged the *in situ* life, but did not effect the integrative response demonstrated by CEM.

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(250) Interaction of Cells with Novel Biodegradable Cryogels in Bioreactors

Bolgen N.¹, Yang Y.², Korkusuz P.³, El Haj A.J.², Pişkin E.¹

¹Hacettepe University, Chemical Engineering Department and Bioengineering Division, Beytepe, 06800, Ankara, Turkey

²Keele University, Institute for Science and Technology in Medicine, Stoke-on-Trent, ST4 7QB, United Kingdom

³Hacettepe University, Faculty of Medicine, Department of Histology and Embryology, Ankara, Turkey

Interaction of cells with novel biodegradable cryogels made of cross-linked HEMA-lactate-dextran with interconnected macropores and with two different pore sizes (large and small) was studied in bioreactors at different regimes (static, perfusion and compression-perfusion). Both an osteoblast like cell line (MG63) and primary cells (bovine chondrocytes) were used in these tests. The samples taken after selected times from the bioreactors were examined by several microscopy techniques (optic, SEM, TEM and confocal). Cell viability and activities were evaluated by alkaline phosphatase (ALP), total protein and DNA assays. Histology of the constructs, which revealed the cells morphology on the surface and within the scaffolds, was also investigated. ALP activity for osteoblast like cells was higher in dynamic conditions (compression-perfusion and perfusion) than in static conditions. The cell culture conditions were found to have a significantly impact not only on the extent of cell attachment and ingrowth but also cell morphology. The chondrocytes proliferated very well in the cryogels demonstrating that the chondrocytes covered fully the scaffold surface after 9-day culture and almost filled the spaces in the pores of the scaffolds after 15 days, at static conditions. Both the osteoblasts and chondrocytes secreted significant amount of extracellular matrices in the scaffolds and the cells subjected to dynamic culture exhibited elongated and highly interconnective morphology. We concluded that these cryogel scaffolds could be excellent candidates for tissue engineering, especially for cartilage tissue regeneration, and related *in vivo* studies are initiated.

(251) Interaction of Hematopoietic Stem Cells with Extracellular Matrix Coated Microcavities

Pompe T.¹, Franke K.¹, Kurth I.¹, Bornhäuser M.², Werner C.¹

¹Leibniz Institute of Polymer Research Dresden, Max Bergmann Center of Biomaterials, Hohe Str. 6, 01069 Dresden, Germany

²Universitätsklinikum Carl Gustav Carus, Med. Klinik I, Dresden, Germany

Bioartificial substrates provide powerful means to trigger stem cell fate decisions by imitating physical and biomolecular cues of tissue specific microenvironments. Towards this aim, we generated a set of surface-engineered model substrates simulating the endosteal niche of the bone marrow to analyse the impact of extracellular matrix (ECM) components and spatial constraints on CD133+ hematopoietic stem cells (HSC). Therefore different ECM components (tropocollagen I, fibronectin, heparin, hyaluronic acid, fibrillar collagen I) were immobilised on poly(ethylene maleic anhydride) coated silicone moulds containing micrometer sized cavities.

In adhesion studies HSC-ECM interaction was investigated on planar ECM glass substrates by reflection interference contrast microscopy to examine the kind and degree of cell-matrix adhesion in a quantitative manner. Strong attachment of HSC was found onto fibronectin with the formation of distinct adhesion areas. Immobilised heparin as well as co-fibrils of collagen I and heparin or hyaluronic acid also induced adhesion, however, with significantly smaller adhesion areas. No adhesion was observed on tropocollagen I and hyaluronic acid coatings. By blocking the $\alpha 5 \beta 1$ -integrin or L-selectin during HSC adhesion to fibronectin or heparin, respectively, the loss of adhesion demonstrated the specificity of the cell-matrix interaction.

FACS analysis and CFU assays in proliferation studies on ECM coated microcavities indicate an adhesion related shift towards granulocyte lineages and a higher fraction of undifferentiated cells on fibronectin surfaces. As time lapse microscopy revealed a preference of HSC for homing to ECM coated microcavities, *in situ* analysis of cell cycle status and cell division inside the microcavities is currently investigated.

(252) Intermittent Loading Accelerates Tissue Development in Engineered Cardiovascular Tissues

Rubbens M.P., Boerboom R.A., Mol A., Bouten C.V.C., Baaijens F.P.T

Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands

Currently used cardiovascular replacements are unable to grow, repair and remodel, which is an important drawback for pediatric patients. Tissue engineering is a promising technique to create living substitutes that are able to adapt to changing conditions. To optimize tissue remodeling and hence improve the mechanical properties, mechanical conditioning strategies are needed. In this study, the effect of mechanical conditioning on the evolution of tissue properties was investigated.

Rectangular strips of PGA coated with P4HB were constrained at the outer edges. The strips were seeded with human myofibroblasts using fibrin as a cell carrier. One week after seeding, half of the samples were subjected to uniaxial intermittent straining (4%, 1 Hz, 3h on/off) using a FlexCell straining device. No external strain was applied to the constrained control samples. Histological and immunohistochemical stainings, matrix analyses and mechanical tests were performed after 2, 3, and 4 weeks of culture ($n = 5$). In addition, multi-photon images were obtained to quantify the collagen orientation and alignment.

The development of tissue properties in engineered constructs was accelerated by intermittent loading. After 4 weeks matrix production and tangent stiffness of constrained and intermittently loaded samples were similar. However, the quality of collagen, expressed by a higher number of collagen crosslinks, and the ultimate tensile strength were increased by intermittent conditioning. Quantification of the collagen architecture revealed alignment of the collagen fibres into the direction of straining.

The developed tools and obtained results will enable us to further optimize strain-based tissue engineering protocols for cardiovascular tissues.

(253) Intracellular Carboxymethylchitosan/Poly(amidoamine) Nanocarriers Loaded with Dexamethasone Enhances Osteogenic Differentiation of RBMSCs *In Vitro*

Oliveira J.M.^{1,2,3}, Kotobuki N.³, Hirose M.³, Mano J.F.^{1,2}, Reis R.L.^{1,2}, Ohgushi H.³

¹3B's Research Group—Biomaterials, Biodegradables and Biomimetics, Department of Polymer Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

²IBB—Institute for Biotechnology and Bioengineering, PT Government Associated Laboratory, Braga, Portugal

³Research Institute for Cell Engineering (RICE), National Institute of Advanced Industrial Science and Technology (AIST), Nakoji 3-11-46, Amagasaki, 661-0974 Hyogo, Japan

Biocompatible and water-soluble carboxymethylchitosan/poly (amidoamine) dendrimer, CMC/PAMAM nanoparticles (6–250 nm in diameter) were synthesized to find applications as a Drug Delivery System (DDS) of dexamethasone (Dex) aimed at modulating the stem cells behaviour namely, proliferation and differentiation both *in vitro* and *in vivo*. In this work, we have demonstrated that these nanoparticles are internalized with high efficiency by different cell types, i.e. cell lines (human osteoblast-like cells, SaOs-2) and primary cultures (Rat Bone Marrow Stromal Cells, RBMSCs) by means of using the fluorescent probe fluorescein isothiocyanate (FITC) and under fluorescence-activated cell sorting (FACS) and fluorescence microscopy (FM) analysis. This work has demonstrated that the FITC-labelled CMC/PAMAM nanoparticles are efficiently internalized by RBMSCs even when loaded with Dex, after a few hours of incubation. Moreover, FACS and FM analysis have shown that the nanoparticles were internalized by RBMSCs in the presence of an endosomal inhibitor (colchicine), which is an indication that internalization mechanism is not exclusively endocytotic. Complementarily, we also assess the osteogenic potential of the Dex-loaded CMC/PAMAM nanoparticles (0.01–1 mg.ml⁻¹) when present in the culture medium and by means of using RBMSCs, *in vitro*. Mineralization studies, ALP quantification, DNA content, Alizarin red and ALP staining were performed to investigate the osteogenic differentiation of RBMSCs (1, 7 and 14 days). Results have shown that the dexamethasone-loaded CMC/PAMAM nanoparticles may find applications in TE, namely as an intracellular DDS since enhanced the osteogenic differentiation of RBMSCs, *in vitro*.

(254) Investigation of a Collagen NanoHA Scaffold with Potential for Bone Tissue Engineering

Cunniffe G., O'Brien F.J., Dickson G.

Tissue Engineering Research Team, Queen's University Belfast, United Kingdom

The objective of this project is to develop a collagen-nanoHA composite scaffold for use in bone tissue engineering. Collagen-based scaffolds have excellent biocompatibility and biodegradability and their pore architecture is ideal to support cellular activity (1).

Hydroxyapatite (HA) is used commonly in tissue-engineered products and can increase scaffold mechanical strength. Problems include resorbability of the mineral by osteoclasts and very brittle resultant constructs. However nano-scale hydroxyapatite particles (nanoHA) are reported as readily resorbable and demonstrate promise when used in composite bone graft substitutes (2).

A number of synthesis methods have been used to create nanoHA particles. The various nanoHA powders produced have been mixed into collagen slurries at quantities ranging between 0–50% wt, followed by freeze drying to produce highly porous scaffolds. The optimal nanoHA/collagen scaffold will be decided upon using a combination of mechanical testing, material analysis and examination of bone cell response on the scaffolds.

To date, mechanical testing of scaffolds has revealed that the scaffold with 50%wt of nanoHA displayed a significantly larger Young's modulus than the control scaffold without nanoHA. Attempts are being made to reduce the size of nanoHA particles in advance of cellular studies. It is thought that this might yield further increases in mechanical properties and encourage cellular proliferation and maturation.

References

1. F.J O'Brien, et al. (2004). Influence of freezing rate on pore structure in freeze-dried collagen-GAG scaffolds, *Biomaterials*; 25: 1077–1086.
2. M.P Ferraz, et al. (2004). Hydroxyapatite nanoparticles: A review of preparation methodologies, *Journal of Applied Biomaterials & Biomechanics*; 2: 74–80.

(255) Investigation of Parameter Limited Range for Operation of a Rotating Wall Vessel Bioreactor

Liu T.Q., Ju Z., Ma X., Cui Z.

Dalian R&D Center for Stem Cell & Tissue Engineering, Dalian University of Technology, China

Rotating wall vessel is one kind of novel bioreactor that possesses grand future in the era of stem cell and tissue engineering. Operation condition and hydrodynamics information in RWV should be deeply understood in order to use it better for high quality cell products with support structures such as microcarriers and microcapsules and/or tissue constructs.

To analyze the forces acting on small tissue pieces or microcarrier particles and to determine the tracks of microcarrier particles in RWV bioreactor, the motion of the microcarrier in the rotating wall vessel (RWV) bioreactor with both the inner and outer cylinders rotating was modeled by numerical simulation. The continuous trajectory of microcarrier particles, including the possible collision with the wall, was obtained. The range of microcarrier radius or tissue size, which could be safely cultured in the RWV bioreactor, in terms of shear stress level, was determined.

In this study, several operation parameters, including rotation speed of the vessel, diameters and densities of constructs suspended in the RWV bioreactor were investigated using the commercial CFD software, FLUENT. The results indicated that the opposite rotation of the two bioreactor vessels can yield better operating results. The range of diameters and density of constructs were also recommended.

(256) Isolation and Characterisation of Human Dental Pulp Stem Cells

Bowen A.¹, Russell H.¹, English A.², Jones E.², Wood S.¹, Kirkham J.¹, Yang X.B.¹

¹Department of Oral Biology, University of Leeds, Leeds, LS2 9LU, United Kingdom

²Academic Unit of Musculoskeletal Disease, St James Hospital, University of Leeds, LS9 7TF, United Kingdom

Introduction: Stem cell therapy and tissue engineering are emerging as potentially powerful treatment strategies. Since everybody experiences tooth loss, the isolation of stem cells from these surgically extracted teeth could provide an alternative human stem cell source without any additional invasive procedures.

Aims: Isolation and characterisation of multipotential stem cells from human dental pulp.

Methods: Primary human dental pulp stem cells (hDPSCs) were isolated using standard organ culture or collagenase digestion methods. Cells were cultured in basal media or under osteogenic, chondrogenic and adipogenic conditions and assessed by colony forming units fibroblastic formation, histology (alkaline phosphatase-ALP, von Kossa, Oil Red O) and biochemical assays (ALP, DNA, sGAG). In addition, dental pulps were divided into six transverse segments in an apical-coronal direction to investigate any site-specific differences in stem cell potential.

Results: hDPSCs demonstrated classic spindle-shaped fibroblast-like morphology. However, hDPSCs showed higher proliferate rate and osteogenic potential compare to human bone marrow mesenchymal stem cells (HBMSCs). Following culture in specialised media, hDPSCs appeared osteogenic, chondrogenic, neurogenic and odontogenic morphologies whereas adipogenesis was low. Cells derived from the middle segments of the pulp demonstrated stronger ALP activity compared with those derived from the more apical and coronal regions. hDPSCs showed similarities with HBMSCs for the expression of some surface markers (such as CD73 and CD105), but not for CD106 and CD13.

Conclusion: This study indicates the potential for using human dental pulp as a source of multipotential stem cells for cell replacement therapies and tissue engineering.

(257) Isolation and Characterization of Cardiac-Derived Progenitor Cells from Human Heart Biopsies

Haag M., Ringe J., Schroeder S., Sittlinger M., Tschoepe C.

Charité Universitätsmedizin Berlin Tissue Engineering Laboratory, Germany

Objectives: Heart failure remains one of the major causes of mortality in the Western world. Owing to the lack of organ donors and complications associated with immune suppressive treatments, scientists are looking for new strategies to regenerate the injured heart. We isolated a new cell type directly from human heart biopsies with cardiac progenitor cell characteristics. The aim of our study was to characterise these cells, and we intend to establish a method to use these cells in tissue engineering for the reconstitution of damaged myocardium *in vivo*.

Methods and Results: Heart biopsies from human donors undergoing a right ventricular endomyocardial biopsy were cultivated. The samples are obtained to investigate the cause of dilatative cardiomyopathy. We established a method to isolate outgrowing cells from these biopsies and studied growth kinetics. To characterise the cardiac-derived cells we used different antibodies including markers for mesenchymal stem cells in flow cytometry, immunohistochemistry and immunofluorescence. The cardiac-derived cells are positive for mesenchymal stem cell like marker and negative for markers of hematopoietic cells. In contrast to other mesenchymal progenitor cells these cells are negative for CD90. Initial analyses demonstrated myogenic potential.

Conclusions: First results indicate that these cells are interesting candidates for the reconstitution of damaged myocardium. Further *in vitro* and *in vivo* experiments are necessary to check the potential of these cardiac-derived progenitor cells.

(258) Isolation, Characterization and Perspective of Clinical Application of Human MMSC's after Skin Burn

Sharifullina S.Z.¹, Chupikova N.I.¹, Yeremenko V.S.², Vasyunina N.Y.¹, Korzhikova S.V.¹, Teplyashin A.S.^{1,2}

¹Institute of Stem Cell, 107031, Kuzneckiy Most str., 17/1, Moscow, Russia

²Beauty-Plaza Medical Corporation, 107031, Kuzneckiy Most str., 17/1, Moscow, Russia

Introduction: The regeneration of skin is provided by means of consecutive differentiation of skin stem cells. The aim of our studies was isolation and characterization of multipotent mesenchymal stromal cells (MMSC's) from human skin, and an estimation of prospective of their application in regenerative medicine.

Methods: MMSC's were isolated by standard method from human dermis (3×5 mm) with the agreement of the patient by dermatome with application of a local anesthesia. The received cells were immunophenotyped by antibodies (BD Biosciences, Germany) to group of surface antigens. For detection of multipotency received cells were differentiated into cells of mesenchymal origin by additional of specifically inductors. For laser dermabrasion of patient's face was used erbium laser UltraFine Er-YAG (Coherent Inc., USA). With the agreement of patients have been generated 2 groups on 5 patients. In experimental group: on 3rd day after operation were applied MMSC's in 3% hyaluronic acid (biomatrix). Then were applied Ung. Solcoseryl (ICN, Germany) up to full epithelization. In control group regeneration of epithelium was spent by standard method.

Results: We isolated cells which had phenotype of MMSC's. At presence of inductors the received cells were differentiated into

cells of a bone, adipose and cartilage tissue. Clinical application of these cells has shown that using MMSC's in biomatrix allows to achieve complete epithelization practically on 5–7 days, and in control group (without cells), 9–12 days.

Conclusions: We derived MMSC's from adult human skin which are a perspective material for regeneration of skin in various fields of reconstructive medicine.

(259) Laminin Peptide Gradient in a Collagen Scaffold shows Guidance Effect on Neurite Growth

Yao L.¹, Damodaran G.¹, Natalia N.¹, Gorman A.², Windebank A.³, Pandit A.¹

¹National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland

²Department of Biochemistry, National University of Ireland, Galway, Ireland

³Department of Neurology, Mayo Clinic, United States of America

Introduction: Type I collagen is suitable for use in neural regeneration applications due to its biological and structural properties. However, the guidance effect on a functionalised collagen scaffold has not been studied before. In this study, collagen scaffolds were cross-linked with microbial transglutaminase (mTGase) and functionalised with either a gradient of laminin peptide (PPFLML LKGSTR) or NGF. The outgrowth of PC12 cells and the guidance effect on neurite growth on these functionalised gradients has been investigated in this study.

Materials and Methods: Type I collagen solution (5 mg/ml) was cross-linked with mTGase and freeze-dried. The collagen scaffold was homogeneously incorporated with laminin peptide (PPFLML LKGSTR) or NGF and freeze-dried. Laminin peptide or NGF gradients were created in the cross-linked collagen scaffolds. The neurite growth of PC12 cells on laminin peptide or NGF incorporated scaffolds were studied by fluorescent (Rhodamine Phalloidin) staining and SEM imaging.

Results: (i) The neurite growth of PC12 cells showed no significant difference on cross-linked or non cross-linked collagen scaffolds, (ii) neurite growth was guided toward the higher level of laminin gradient and laminin incorporation had no significant effect on the length of neurite outgrowth, (iii) neurite growth on NGF conjugated collagen scaffold showed a dose dependent behavior.

Discussion: The enzymatically cross-linked collagen scaffold provided a structural support for PC12 cell differentiation and neurite outgrowth. Laminin gradient created on the cross-linked collagen scaffold can function as a guidance cue for neurite orientation. A NGF functionalized collagen scaffold stimulates neurite growth.

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(260) Liver Tissue Engineering using an Acellular Whole Organ Bioscaffold with Perfusion Cell Seeding

Baptista P.M., Atala A., Soker S.

Wake Forest Institute of Regenerative Medicine, Wake Forest University Health Sciences, Winston-Salem, NC, United States of America

Our laboratory was recently able to use a decellularization method to generate an organ bioscaffold from a whole liver, while preserving its functional vascular network. These bioscaffolds offer the proper macro and micro architecture and environmental cues to liver cells seeded in the bioscaffold. The purpose of this study was to investigate the feasibility of re-cellularizing the whole organ bioscaffold with two cell types using a perfusion method.

Mouse GFP labeled endothelial cells and human hepatocyte committed progenitors—HepG2 cells—were seeded through the cannulated portal vein of the bioscaffold. The seeded bioscaffolds remained in a bioreactor system with permanent culture medium perfusion for one week. Fluorescence microscopy was used to image the endothelial cells and to determine cell density and seeding efficiency. Immunohistochemistry and TUNEL assay were used to identify the engrafted cells and to determine cell proliferation and apoptosis.

The perfused endothelial cells attached and formed a monolayer on the luminal side of the vascular channels. HepG2 cells showed homogeneous seeding and high cell density throughout the bioscaffold (1–1.5 cm thickness). Immunohistochemistry showed progressive tissue formation and organization. Cellular proliferation with minimal apoptosis has also been observed within the bioscaffold.

Our results demonstrate that a novel acellular liver-derived whole organ bioscaffold can be re-cellularized using a perfusion cell seeding process in a bioreactor. Cell proliferation and reduced levels of apoptosis were associated with three-dimensional tissue formation and organization *in vitro*. This technology may provide a new approach for liver organ engineering, critical for the treatment of terminal liver disease.

(261) Mathematical Modelling of Growth Factor Induced Bone Regeneration: Importance of Angiogenesis

Van Oosterwyck H., Vander Sloten J., Geris L.

K.U. Leuven, Division of Biomechanics and Engineering Design, Leuven, Belgium

Introduction: Angiogenesis is a prerequisite for bone regeneration and bone engineering. A novel mathematical model of bone regeneration, incorporating angiogenesis, was developed and applied to simulate tissue engineering strategies for the treatment of atrophic non-union.

Methods: An existing bioregulatory model for fracture healing [1] was extended with expressions for angiogenesis and its relation to bone forming processes. Vascular growth factors, endothelial cells and vascular matrix were defined as additional variables, and were related to chemotaxis, osteogenic differentiation and chondrocyte hypertrophy. An atrophic non-union model [2] was considered and tissue engineering treatment strategies were simulated, including the administration of mesenchymal stem cells (MSCs), osteogenic and/or angiogenic growth factors.

Results: No cartilage or bone was predicted over 12 weeks time, while vascularisation continued progressively, as experimentally

observed [2]. Administration of only MSCs restored healing if administered in the callus at fracture initiation, and at a sufficiently high concentration (20,000 cells/ml). When administered at post fracture day 20, healing could only be resumed if osteogenic and angiogenic growth factors were administered simultaneously.

Discussion: A mathematical model of bone regeneration, including angiogenesis, was developed and applied to study tissue engineering strategies for the treatment of atrophic non-union. It may be used as a computational tool to help define additional experiments on the effect of different treatment modalities.

References

1. A. Bailón-Plaza, M.C.H. van der Meulen (2001) *J Theor Biol* 212:191–209.
2. H.C. Brownlow, A. Reed, A.H.R.W. Simpson (2001) *Injury* 32:519–524.

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(262) Measurement of Glucose and Oxygen Concentration in Biomimetic Constructs

Alp B.¹, Rong Z.², Cheema U.¹, MacRobert A.J.³, Vadgama P.², Brown R.A.¹

¹University College London, Tissue Repair and Engineering Centre, Institute of Orthopedics, Stanmore, London, HA7 4LP, United Kingdom

²Centre for Materials Research, Queen Mary, University of London, London, E1 4NS, United Kingdom

³National Medical Laser Centre, 67 Riding House Street, W1P 7NN, United Kingdom

Monitoring nutrient, oxygen transport and cell viability is crucial to understand cell growth in tissue engineered constructs. The main concern in 3D tissue constructs is rapid cell death in the centre of the constructs because of diffusion limitations. We have established a highly controllable method to monitor glucose and oxygen at the centre of dense 3D spiral collagen constructs suitable for investigation of bioreactor operation. Acellular and human dermal fibroblast seeded collagen constructs (2×10^6 cells/construct) were prepared by plastic compression technique. Single compressed (SC) constructs were rolled to spirals and compressed second time to further increase collagen density (double compression-DC). Glucose and oxygen were measured by electrochemical and fluorescent optical-fibre probes respectively. Surface/core cell viability were analyzed together with levels of VEGF gene expression (by quantitative PCR).

Nutrient transfer is controlled by different factors; diffusion distance, cell density and scaffold material properties. Cell and collagen densities in DC constructs were 3 fold greater than SC constructs. Core glucose and oxygen levels fell rapidly with cells metabolizing, with levels mainly dependent on cell density. At 5 days cell viability was decreased from surface to centre in DC constructs (65–70%) but not in SC constructs. Increased VEGF gene expression was related to prolonged cell exposure to core O₂ levels. This model shows that high density collagen constructs enable

good perfusion (>1 mm). Angiogenic signaling was stimulated without the need for either true hypoxia or substantial cell death.

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(263) Measurement System Capability: A Comparison of Manual and Automated Cell Counting Systems

Liu Y., Houd P., Williams D.

Wolfson School Mechanical and Manufacture Engineering, Loughborough University, Loughborough, LE11 3TU, United Kingdom

Cell concentration and cell viability are two of the most important productivity measurements in cell culture related production and research. A short term measurement system capability study is presented to establish the relative capability of manual (haemocytometer) and automated (Cedex) cell counting systems for investigating process improvement opportunities in a nascent cell culture process.

Both measurement processes were challenged using selected surrogate samples. Five concentrations of latex beads were prepared, 1×10^5 /ml, 3×10^5 /ml, 5×10^5 /ml, 7×10^5 /ml and 1×10^6 /ml, to give five batches or parts representing the operating range of cell concentrations during a typical adherent cell culture process. Four identical replicates were prepared from each homogenous solution of beads (parts). Four operators were selected and all measurement procedures were carried out according to the established standard operating procedures. All data were analysed using MiniTab software v14 (Minitab, UK).

Gauge R&R analysis indicated that for both measurement systems most of the variation was due to differences between parts. For the manual and automated systems, the measurement system error consumed 8.0% & 4.9% of the total variation respectively. For the manual system the measurement error was composed of both repeatability (6.78%) and reproducibility (1.2%) error. Whilst for the automated system it was composed of only repeatability error. Further analysis showed that the automated system could discriminate at least 6 product categories (number of distinct categories) whereas the manual system could discriminate at most 4 categories in the same operational region.

This study demonstrates that both systems were capable of detecting product variation, but the automated cell counting system had greater relative capability than the manual system under the current operating conditions.

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(264) Mechanical Loading Modulates Calcium Signalling Within Mesenchymal Stem Cells

Campbell J.J., Bader D.L., Lee D.A.

School of Engineering and Materials Science, Queen Mary University of London, Mile End Road, London, E1 4NS, United Kingdom

Mesenchymal stem cells (MSCs) provide a promising cell source for tissue-engineered repair strategies due to their expansion and multi-lineage differentiation potential. In addition, increasing evidence confirms the role for the mechanical environment in maintaining and enhancing extracellular matrix synthesis, with intracellular calcium signalling implicated as a mediator in mechanotransduction pathways. Accordingly, we tested the hypothesis that static mechanical strain influences calcium signalling in MSCs.

Human MSCs cultured to passage 5 were encapsulated in alginate constructs. The specimens were stained with the fluorescent Ca^{2+} indicator Fluo-4 and placed between the platens of a compression rig mounted on an inverted microscope fitted for confocal microscopy. Ca^{2+} fluorescence was monitored throughout two consecutive 20 minute periods, during the second of which experimental specimens were subjected to a 20% static uniaxial compressive strain. Control constructs were observed during two unstrained periods. The use of image analysis software allowed continuous monitoring of multiple cells within the same field of view, and cell-tracking between both analysis periods allowed for a pair-wise approach to data analysis.

Spontaneous intracellular calcium signalling was observed within a subset of MSCs. Distinct sub-populations of cells up-regulated or down-regulated the frequency of calcium transients during the application of static strain. These values were statistically significant ($p < 0.05$) by χ^2 test of independence. This variable response highlights the heterogeneous nature of MSC populations, a finding which may have important implications for their successful use in cell therapies.

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(265) Mechanical Loading Triggers Cortical Actin Disassembly in Articular Chondrocytes

Campbell J.J., Knight M.M.

School of Engineering and Materials Science, Queen Mary University of London, Mile End Road, London, E1 4NS, United Kingdom

Mechanical loading is necessary for homeostasis in a wide variety of tissues although the process of mechanotransduction is poorly understood. The actin cytoskeleton comprises a dynamic structure, which has been implicated in integrin-mediated mechanotransduction whilst also providing the cell with its mechanical integrity. This study tests the hypothesis that mechanical loading modulates actin dynamics and organisation in articular chondrocytes.

Chondrocytes were transfected with an eGFP-actin plasmid and seeded in 3% agarose constructs. After 24 hrs in culture, constructs were subjected to cyclic compression (10 cycles, 1 Hz, 0–15% strain) on the stage of an inverted confocal microscope. A Fluorescence Recovery After Photobleaching (FRAP) protocol was then performed in which the GFP-actin was photobleached in 4 separate regions ($2 \times 2 \mu\text{m}$) positioned around the cell cortex. Fluorescence recovery was measured over the subsequent 10 minute period. Normalised FRAP curves were modeled by fitting a two phase exponential.

Transfected chondrocytes exhibited dense cortical GFP-actin, reflecting the distribution on F-actin observed in non-transfected chondrocytes in agarose and cartilage explants. Cyclic compression resulted in cell deformation followed by significantly reduced

FRAP compared to unloaded controls ($K1$, $p < 0.05$). No significant differences were found between different cortical regions in loaded cells, suggesting global modulation of actin dynamics. Analysis of non-bleached regions revealed a significant reduction of cortical GFP-actin intensity over the 10 minute period.

These results suggest that mechanical loading triggers depolymerisation of cortical F-actin, possibly involving intracellular calcium signalling. Although the reason for this mechanosensitive actin remodeling is unclear, it is likely to have major implications for cell mechanics and mechanotransduction.

(266) Mechanical Stimulation of Osteoblasts by Steady and Dynamic Fluid Flow

Jaasma M.J., Plunkett N.A., O'Brien F.J.

Department of Anatomy, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2, Ireland
Trinity Centre for Bioengineering, Trinity College, Dublin 2, Ireland

In bone tissue engineering, flow perfusion bioreactors can be used to mechanically stimulate osteoblasts, resulting in increased bone matrix formation [1]. Steady and dynamic fluid flow can have different effects on osteoblast activity in 2D culture [2]. Therefore, the goal of this study was to investigate the effects of steady, pulsatile, and oscillatory fluid flow on osteoblast activity within a 3D collagen-glycosaminoglycan scaffold.

Collagen-glycosaminoglycan scaffold discs [3] were seeded with 2×10^6 MC3T3-E1 osteoblasts. After six days of pre-culture, constructs were separated into five groups: 1) Steady flow (1.0 mL/min), 2) Pulsatile flow (peak rate = 1.0 mL/min), 3) Oscillatory flow (peak rate = 1.0 mL/min), 4) Low flow control (steady flow, 0.05 mL/min) and 5) Static culture. After 25 hr of bioreactor [4] or static culture, constructs were assessed for cell number via DNA quantification and expression of cyclooxygenase-2 (COX-2), Collagen I, and osteopontin (OPN) via real-time RT-PCR and normalisation to 18-S rRNA expression.

Steady and pulsatile flow resulted in 13- to 28-fold higher COX-2 expression compared to static culture ($p < 0.03$). Steady flow also produced a 130% increase in OPN expression over static culture and oscillatory and pulsatile flow ($p < 0.005$). These results indicate that for short-term culture, steady flow provides the greatest mechanical stimulation of osteoblasts in a 3D environment. Analysis at additional culture durations is in progress.

References

1. Bancroft GN. (2002). PNAS 99:12600.
2. Jacobs CR. (1998). J Biomech 31:969.
3. O'Brien FJ. (2004). Biomaterials 25:1077.
4. Jaasma MJ. (2006). J Biomech 39:S218.

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(267) Mesenchymal Adipose Cells Growing in Ammiomax Culture Medium Survive Long Term with a Stable Phenotype

Trejo C.G., Gomez-Gil V., Perez K.B., Rodriguez M., Corrales C., Bujan J.

Department of Surgery and Medical Specialities, University of Alcalá, Madrid, Spain

Mesenchymal cells are multipotential and able to self-renew and to differentiate into different lineages. During this stage, they express embryonic stem cells markers as SOX-2, Nanog and Oct-3/4. The aim of this study was to test these transcription factors in our Mesenchymal Adipose-Derived Stem cells (MADS).

MADS were isolated from rat inguinal fat and cultured in Amniomax[®] (Gibco) during more than 180³ passages. We analyzed the immunohistochemical expression of the different markers: Nanog, Oct-3/4 and SOX-2. Proliferation studies were performed in first passage cells during 15 days by flow cytometry.

The three markers appeared during all the passages. The average percentage of Oct-3/4 was about a 60%; SOX-2, 45%; and Nanog, 27%. Cells from first passage showed a high proliferation rate 206 times higher than the initial population.

If cells isolated from rat adipose tissue and cultured in Amniomax are able to survive for more than 175 passages, showing a high proliferation rate and the expression of embryonic stem cells markers in a part of the population during all the passages, they can represent a stable culture source to be employed in tissue engineering.

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(268) Mesenchymal Stem Cell Proliferation and Metabolic Profiling in Spinner Flasks

Higuera-Sierra G.¹, Janssen F.^{1,2}, Van Dijkhuizen-Radersma R.², Van Boxel A.³, Van Blitterswijk C.¹

¹Department of Tissue Regeneration, Institute for biomedical Technology, University of Twente, Postbus 217 7500AE Enschede, The Netherlands

²R&D Division, Progentix BV. Prof. Bronkhorstlaan 10-D 3723 MB Bilthoven, The Netherlands

³Systems and Control Group, Dept. ATV—AFSG—SCO, Wageningen University, P.O. Box 17, 6700 AA, Wageningen, The Netherlands

Proliferation of Human Bone Marrow Mesenchymal Stem Cells (HBMSC) in 2D systems is not optimal for achieving high cell numbers (e.g. 200–800 million cells). In order to expand HBMSC to significant numbers in a closed and controlled environment, we are developing a bioreactor system involving a spinner flask containing microcarriers.

We tested different stirring rates and feeding regimes with respect to viable cells. In addition, we obtained a metabolic profile including glucose, lactate, glutamine and ammonia for HBMSC under the different conditions mentioned above.

Our data suggests that in the range of stirring rates used (10–30 RPM), there are little differences in growth rate, metabolite consumption and production. Independent of the stirring rate, the data shows that HBMSC enter a stationary phase after a few days in culture. By adding 30% working volume of microcarriers and medium, HBMSC did not enter a stationary phase and proliferated linearly for 14 days.

Interestingly, HBMSC proliferation rate continues to be higher under static than under dynamic conditions. This is reflected by the

HBMSC growth rates (μ) in the two systems: 0.04 hr⁻¹ in T-flasks and 0.01 hr⁻¹ in spinner flasks.

We are developing a mass balance model from metabolite and viable cell concentration measurements obtained. Matlab[®] functions allow us to determine quantitative differences on growth profiles. From the model, we expect to obtain the growth kinetics and metabolic rates of HBMSC under different conditions.

(269) Mesenchymal Stem Cells and Tensile Strain

Glossop J.R., Cartmell S.H.

University of Keele, Institute for Science and Technology in Medicine, Guy Hilton Research Centre, Hartshill, Stoke-on-Trent, Staffordshire, ST4 7QB, United Kingdom

Human mesenchymal stem cells (MSCs) have potential applications in tissue engineering because they are multipotent, unspecialized cells which can differentiate into numerous different lineages. Evidence indicates that tensile strain can have multiple effects on cell responses, including proliferation, alignment and gene expression. However, there is limited data describing its affect on MSCs. We investigated the effects of tensile strain application on gene expression in primary human MSCs.

MSCs (Lonza) were grown in monolayer on pronectin-coated 6-well tissue culture plates (Flexcell). Cells were cultured at 5×10^4 cells/well in DMEM supplemented with 10% FCS, 1% L-glutamine and 1% antibiotics. Uniaxial tensile strain, equivalent to 1 and 3% cell elongation, was applied to cells for 1 hour using a FX-4000T system (Flexcell), and samples were taken 2 hours subsequently. Isolated total RNA samples were used for microarray analysis using Affymetrix Human Genome U133 Plus 2.0 arrays (performed by Almac Diagnostics). Real-time PCR was performed to validate genes of interest identified through microarray analysis.

Microarray analysis revealed >250 genes were differentially expressed compared to control, for both 1 and 3% cell elongation. In each case, approximately 25% of the genes were up-regulated. In both conditions, expression of aurora kinase A interacting protein 1 (a negative regulator of mitosis) was up-regulated the greatest, approximately 5-fold. Several genes relating to actin binding were also found to be differentially expressed in both conditions.

Our data indicate that MSCs exposed to tensile strain have altered gene expression profiles that indicate a transition away from proliferation.

(270) Mesenchymal Stem Cells as Substitutes for Schwann Cells

Mahay D., Terenghi G., Shawcross S.

The University of Manchester, Blond McIndoe Research Laboratories, Manchester, M13 9PT, United Kingdom

Schwann cells (SCs) provide physical support and guidance for peripheral nerve regeneration following injury. *In vitro* these supporting cells are slow growing, hence not well suited to a tissue engineering approach to nerve repair. Adult rat bone marrow mesenchymal stem cells (MSCs) were differentiated into SC-like cells using an established cocktail of growth factors: glial growth factor-2, basic fibroblast growth factor and platelet derived growth factor.

Semi-quantitative RT-PCR, Western blotting and immunocytochemistry approaches were used to detect the expression of glial cell markers and neurotrophic factors in MSCs in comparison with SCs (positive control). The presence of the glial markers, calcium binding protein S100, glial fibrillary acidic protein, low affinity neurotrophin receptor p75 and the intermediate filament protein nestin, was investigated. Also investigated were nerve growth factor, brain derived neurotrophic factor, glial derived neurotrophic factor, leukaemia inhibitory factor, ciliary neurotrophic factor, neurotrophin-3 and neurotrophin-4. Differentiated MSCs expressed transcripts of all the above glial markers, nerve growth factor, brain derived neurotrophic factor, glial derived neurotrophic factor and leukaemia inhibitory factor. Similarly, differentiated MSCs like SCs immunostained positively for S100, glial fibrillary acidic protein, p75, brain derived neurotrophic factor and glial derived neurotrophic factor. The results of the study provide evidence that SC-like cells have similar morphological and phenotypic characteristics to SCs, and are likely to be suitable candidates for peripheral nerve repair.

(271) Mesenchymal Stem Cells Maintain a Glycolytic Metabolism under Normoxic Conditions

Pattappa G., De Bruijn J.D., Lee D.A.

School of Engineering and Materials Science, Queen Mary University of London, Mile End Road, E1 4NS, United Kingdom

Mesenchymal stem cells survive *in vivo* under oxygen levels between 4–7% and their proliferation and differentiation is influenced by the oxygen tension. To understand this effect and predict cell behaviour within 3D culture systems, the study investigated the oxygen consumption, glucose consumption and lactate production by human mesenchymal stem cells.

Human mesenchymal stem cells (Cambrex, UK) were used between passages 2–4. 130 microlitre aliquots of cell suspensions containing 5×10^4 , 1×10^5 , 1.5×10^5 and 2×10^5 cells were added to individual wells of a 384 oxygen biosensor well plate (BD Biosciences, Oxford, UK) and separate 384 well plates. Plates were sealed with the biosensor plate placed in a fluorometer (Fluostar Galaxy, BMG, Aylesbury UK) set at 37°C for the measurement of oxygen consumption. The remaining plates were placed in a standard tissue culture incubator and medium was removed from the well plates at 30 minutes, 1, 2, 4 and 6 hour time points for measurement of glucose and lactate.

No significant differences were observed in the mean per cell oxygen and glucose consumption rates and per cell lactate production rate between passages 2–4. At passage 3 and for wells containing 1.5×10^5 cells showed oxygen consumption rates of 52 ± 4.8 fmoles/hr/cell, glucose consumption rate was 342.1 ± 33.5 fmoles/hr/cell and lactate production rate was 839.6 ± 79.3 fmoles/hr/cell. The production of lactate suggests that the cells are preferentially glycolytic, despite the presence of oxygen, a phenomenon known as the Warburg effect.

(272) Mesothelial Cells as Origin of Abdominal Adhesions

Gomez-Gil V., Garcia-Hondurilla N., Corrales C., Trejo C.G., Rodriguez M., Bellon J.M.

Department of Medical Specialities and Surgery, Faculty of Medicine, University of Alcalá. Alcalá de Henares, Madrid, Spain

Nature of cells involved in adhesion formation after abdominal surgery is not clear. Our aim was to study phenotype and proliferation of cells forming adhesion tissue.

Adhesions collected 7 days after abdominal surgery were used to establish cell cultures using omentum as control. Cells were cultured in Amniox medium and examined by immunofluorescence: α -sma, vimentin, desmin, myosin, E-cadherin, PODXL, SSEA-4, SSEA-1, Oct-3/4, CD9, CK5 and fibronectin. Cell proliferation assays were performed by trypan blue and flow cytometry.

Adhesion cells showed different phenotypical patterns, correlating with histology, allowing us to differentiate between mostly adipose and mostly fibrotic adhesions. About 65% of cells from generally adipose adhesions showed mesenchymal markers and 23% desmin, compatible with vascular evolution. Otherwise, cells from fibrotic adhesions showed a myofibroblastic pattern. Mesothelial cells from omentum expressed CK5 (85%), E-cadherin, SSEA-4 and PODXL (100%). All the populations were Oct-3/4(+). Proliferation rate was higher in adhesions than in omentum.

1) Culture medium (Amniox) conditions cellular survival, proliferation rate and phenotype; 2) Adhesion cell phenotype depends on the content of adipose/fibrotic areas in tissue samples; 3) Undifferentiated cells from omentum could be the responsible of different cell lineages found in adhesions. 4) So they could be useful as a source in several tissue engineering applications.

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(273) Methods for Routine and Reproducible Scaffold Characterisation

Mather M.L., Crowe J.A., Morgan S.P., White L., Tai H., Shakesheff K.M.

University of Nottingham, School of Electrical and Electronic Engineering & School of Pharmacy, University Park, Nottingham, NG7 2RD, United Kingdom

Tissue scaffolds are integral to many regenerative medicine therapies providing suitable environments for tissue regeneration. In order to assess their suitability, methods to routinely and reproducibly characterise scaffolds are needed. These will facilitate the implementation of quality control in scaffold fabrication, enable the investigation of process capability and provide metrics to compare scaffold characteristics with performance.

Scaffold structures are typically complex, and thus their characterisation is far from trivial. This is demonstrated by the lack of consensus in the literature on both the parameters that should be used and suitable strategies for their measurement. For example many studies cite porosity as being a key parameter (1); however whilst this is an important property it is not without ambiguity as structurally different scaffolds can have identical porosities.

This work demonstrates the capabilities of different imaging modalities and analysis techniques used to characterise scaffolds fabricated from poly(lactic acid) (PLA) using supercritical carbon dioxide. Three structurally different scaffolds were used. The scaffolds were imaged using: scanning electron microscopy; x-ray computed tomography; magnetic resonance imaging; light microscopy

and terahertz imaging. In each case two dimensional images were obtained from which scaffold properties were determined using image processing.

The findings of this work highlight how the chosen imaging modality and image processing technique can influence the results of scaffold characterisation.

Reference

1. Heang Oh, S. et al. (2007). *in vitro* and *in vivo* characteristics of PCL scaffolds with pore size gradient fabricated by a centrifugation method. *Biomaterials*. 28:1664–1671.

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(274) Micro and Nanostructure Evolution Study of Novel Injectable Calcium Phosphate Cements Prepared by Ceramic and Sol-Gel Processes

Mestres G., Castaño O., Navarro M., Almirall A., Ginebra M.P., Planell J.A.

Biomaterials, Implants and Tissue Engineering Division, IBEC, Barcelona, Spain

Biomaterials, Biomechanics and Tissue Engineering Group, ET-SEIB-UPC, Barcelona, Spain

Alkaline doped calcium phosphate cements (CPCs) are very promising and useful precursors to obtain hydroxyapatite-like injectable implant compounds via fast hydrolysis and precipitation [1]. In this sense $\text{Ca}_2\text{NaK}(\text{PO}_4)$ (CSPP) powders were synthesized with a modified Pechini protocol and compared with the ceramic based conventional cement preparation method. Pure CSPP was obtained between 800°C and 1200°C. The characteristics of the Pechini cements were evaluated by X-ray diffraction (XRD), scanning electron microscopy (SEM) and laser particle size analyzer among others. Results were then compared with powders prepared by the ceramic method. The focus was maintained on mechanical properties, to understand how compression resistance relates to the micro and nanostructural changes and morphology. Particle size, cement homogeneity and reaction rate influenced the behavior of the cement in both dry and saline immersed conditions. Several samples synthesized by the two methods were immersed in aqueous solutions at various time points to evaluate how environmental media determines cement features, particularly on their surface.

Reference

1. F.C.M. Driessens, M.G. Boltong, E.A.P. de Maeyer, R. Wenz, B. Nies and J.A. Planell. (2003). The Ca/P range of nanoapatitic calcium phosphate cements. *Biomaterials* 23 pp. 4011–4017.

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(275) Microfibrous Vascular Scaffold with Controlled Fabrication and Function

Andrews K.D.¹, Feugier P.², Black R.A.¹, Hunt J.A.¹

¹UKBioTEC, Division of Clinical Engineering, University of Liverpool, L69 3GA, United Kingdom

²Vascular Surgery Unit, Hôpital E. Herriot, University Cl Bernard Lyon1, Lyon, 69 437, France

An ideal solution to blood vessel replacement is continually sought. Such scaffolds require suitable surface and mechanical properties, also maintaining cellular function, whilst exposed to shear stresses. The aim of this work was to investigate the potential of electrostatically spun scaffolds, in comparison to clinically used vascular materials, to control these criteria.

Three vascular materials were characterised using SEM: Dacron[®], ePTFE and electrostatically spun polyurethane scaffolds (Tecoflex[®] SG-80A) (PU). Ethylene oxide sterilised, collagen-impregnated material discs were seeded with HUVECs (9.5×10^5 cell density for 7 days), then exposed to shear stresses of 1 or 2 Pa for 1 hour. Cell coverage, spreading and orientation from static and shear-exposed samples, cytoskeletal involvement and cell profiles were examined. Immunohistochemistry for a range of extracellular matrix and adhesion markers was analysed.

All scaffold forms were determined through the fabrication conditions. PU scaffolds produced the greatest cellular responses for shear-adaptation, with well spread cells spanning the fibres, highly defined F-actin fibres, profuse focal contacts, high levels of adhesion markers and flat height profiles. Cell retention after shear exposure on PU was greater in comparison to the other materials. Further investigation of the spun PU scaffolds revealed their controlled structure and topography to be significant in affecting and stimulating cellular behaviour. The scaffold properties were also significantly linked to differences in the adhesion mechanisms, with high upregulation of extracellular matrix molecules correlating with increased topographical features.

Electrostatically spun PU scaffolds showed significant potential as controllable scaffolds, at both fabrication and function stages, for vascular tissue engineering.

(276) Micro-Macro Coupling Approach of Bone Growth within Non-Reabsorbable Scaffolds

Sanz J.A., García-Aznar J.M., Doblaré M.

University of Zaragoza, Spain

Bone tissue regeneration using scaffolds is receiving an increasing interest in orthopedic surgery and tissue engineering applications. The problem in mathematical and computational terms inherently attends to different length scales (cell, scaffold, tissue) as well as different time scales. In this work, we present the implementation of the asymptotic homogenization theory (following Terada et al., 1998) to simulate the macroscopic scale (tissue level) and the microscopic one (pore level) as well as their interaction. In this context and by means of a bone regeneration model, bone growing is numerically simulated within a bioresorbable scaffold implanted in the distal femoral condyle of a rabbit. Bone growing scheme and evolutive resorption of the scaffold microstructure is greatly simplified by using the voxel finite element method into a representative volume element of the scaffold microstructure (Adachi et al., 2006). Results are presented in the macro-geometry 3D, and in the scaffold microstructure at several locations of the scaffold macroscopic mesh. Therefore, presented results concern to remodelled bone in the surrounding of the scaffold and newly regenerated

bone, in the macro-geometry and scaffold microstructure, respectively.

References:

1. Terada K, Ito T, Kikuchi N. Characterization of the mechanical behaviours of solid-fluid mixture by the homogenization method. *Comp. Meth. in Appl. Mech. and Eng.*, 1998, vol. 153, pp. 223–257.
2. Adachi T, Osako Y, Tanaka M, Hojo M, Hollister SJ. Framework for optimal design of porous scaffold microstructure by computational simulation of bone regeneration. *Biomaterials*, 2002, vol. 27, pp. 3964–3972.

(277) Micro-Nano Composite Mesh Scaffold in Mouse MPC Osteogenic Differentiation

Martins A., Pinho E.D., Malafaya P.B., Marques A.P., Reis R.L., Neves N.M.

3B's Research Group—Biomaterials, Biodegradables and Biomimetics; University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

IBB—Institute for Biotechnology and Bioengineering, Braga, Portugal

In the biomaterials field, nanofibrous structures and composites are promising materials to produce scaffolds mimicking the architecture of the extracellular matrix (ECM). Indeed, the ECM of connective tissues is a biological example of a micro-nanofibrous structure. The main objective of this work is to evaluate a novel scaffold produced with microfibrils obtained by melt extrusion of a blend of chitosan and a synthetic biodegradable polymer, poly (butylene succinate) (50:50 wt). This blend was also compounded with electrospun chitosan nanofiber meshes (0.05% wt) to obtain a nano-reinforced composite.

Tensile mechanical testing of the reinforced microfibrils showed that the modulus is increased up to 70% (295.7 ± 16.2 MPa). A significant increment of water uptake and weight loss was also observed. This result was accomplished most probably by the significant increase of the microporosity of reinforced microfibrils, as analysed by microCT. It is hypothesized that the combination of improved mechanical properties and also degradability of the developed fibres do not deter the excellent biological performance of 3D mesh scaffolds produced by fibre bonding.

Mouse bone marrow-derived mesenchymal progenitor cells (mMPCs) were seeded on those 3D scaffolds and induced to differentiate into the osteogenic lineage. The adhesion, morphology and distribution of differentiated mMPCs were analysed by SEM and fluorescence microscopy. Viability and proliferation assays demonstrated cell activity similar to that observed in scaffolds obtained with microfibrils without reinforcement. The osteogenic genotype of differentiated mMPCs was confirmed by Real Time-PCR.

It was concluded that the reinforced microfibre meshes also sustain mMPC osteogenic differentiation and are adequate for bone tissue engineering.

(278) Migration Promotes Myoblast Proliferation by Reducing Myotube Formation

*Chowdhury S.R., Muneyuki Y., Kino-oka M., Taya M.

*Osaka Univ, Dept Biotechnology, Osaka 5650871, Japan; Osaka University, Div Chemical Engineering, Osaka 5608531, Japan

Recently, cultured myoblast cells from autologous skeletal muscles have been implanted to human for treating myocardial infarction, associated with dysfunction of cardiomyocytes and irreversible cell loss, to improve cardiac activity. But the expansion of myoblast cells for transplantation is still challenging due to the lower growth potential accomplished with myoblast differentiation during *in vitro* culture. In this study we attempted to improve the migration of myoblast cells for promoting their proliferation.

Myoblast cells were cultured in DMEM (containing 10% FBS), with or without EGF, on plain and laminin-coated surfaces. The proliferative cells were detected using BrdU. To estimate the migration rate and the disjunction time after cell division, dynamic cellular behavior was evaluated using a time-lapse observation tool.

Myoblast cells cultured with EGF-supplemented DMEM on laminin-coated surface increased the migration rate approximately 2.7 and 1.4 times, compared with those in EGF-free DMEM on plain and laminin-coated surface, respectively. It was observed that higher migration facilitates cellular disjunction, which is release of cells from intercellular contact, just after cell division. The average disjunction time after cell division was significantly lower under the culture conditions with higher migration rate than that with lower migration rate, and disjunction time was found to decrease with increasing migration rate. In addition proliferative cells also increased under the culture conditions with higher migration rate. These results demonstrate that synergy effects of laminin and EGF facilitate myoblast migration that enhanced cellular disjunction after cell division and thereby myotube formation was reduced, resulting is the improvement of growth potential.

(279) Minimal BMP-2 Concentration Inducing Osteogenic Differentiation in a Mouse Myoblast Cell Line (C2C12) and Ectopic Bone Formation in Rats

Kloesch B., Hoering B., Banerjee A., Dopler D., Van Griensven M., Redl H.

Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Centre, Austrian Cluster for Tissue Regeneration, Vienna, Austria

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor beta (TGF- β) superfamily. *In vivo* application of recombinant BMPs plays an important role in bone healing of critical size defects and tissue engineering. Little is reported about the minimal dose of BMP-2 responsible for inducing osteogenic differentiation of myoblasts and ectopic bone formation of clinically approved hBMP-2 (Wyeth) in *in vitro* and *in vivo* studies.

Therefore our aim was to evaluate the minimal effective dose of hBMP-2 that induces osteogenic differentiation in a mouse myoblast cell line (C2C12) and ectopic bone formation in a rat model.

Briefly, C2C12 cells were incubated for 5 days with different concentrations of hBMP-2 (10–500 ng/ml). Osteogenic differentiation was evaluated by measuring ALP activity and RT-PCR for osteogenic marker genes like ALP, Osteocalcin, Runx2, Osterix and PTHR1.

A dose of 100 ng/ml hBMP-2 already induced ALP, Osteocalcin and Runx2 expression, however, a dose of 10 ng/ml was sufficient to induce Osterix and PTHR1 expression.

For our *in vivo* studies Collagraft blocks as a carrier were loaded with different doses of hBMP-2 (0.01–2 µg) and implanted into dorsal muscle pockets of Sprague Dawley rats. After 4 weeks animals were sacrificed and ectopic bone formation was evaluated by measuring ALP activity, RT-PCR (ALP, Osteocalcin, Osteopontin, Bone Sialoprotein, Runx2, Osterix and PTHR1) and by µCT.

Our results showed that a single dose of 250 ng hBMP-2/implant was sufficient to induce ectopic bone formation in our rat model.

These data showed that low doses of hBMP-2 are sufficient for inducing osteogenic differentiation of myoblasts and ectopic bone formation.

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(280) Modelling of Human Bone Marrow Mesenchymal Stem Cell Kinetics

Higuera-Sierra G.¹, Janssen F.^{1,2}, Van Dijkhuizen-Radersma R.², Van Bostel A.³, Van Blitterswijk C.¹

¹Department of Tissue Regeneration, Institute for biomedical Technology, University of Twente, Postbus 217 7500AE Enschede, The Netherlands

²R&D Division, Progentix BV. Prof. Bronkhorstlaan 10-D 3723 MB Bilthoven, The Netherlands

³Systems and Control Group, Dept. ATV-AFSG-SCO, Wageningen University, P.O. Box 17, 6700 AA, Wageningen, The Netherlands

Human bone marrow mesenchymal stem cells (HBMSC) are at the core of different tissue engineering strategies. HBMSC proliferation is important to make clinical applications viable. At present, the quantification of processes involved in HBMSC proliferation is not defined. This work involves the quantification of HBMSC proliferation and metabolic profiling *in vitro*.

We cultured three HBMSC-passage-3 donors under batch conditions for 7 days. We measured metabolites involved in major metabolic pathways (i.e. glucose, lactate, glutamine, glutamate and ammonia). Also, from the t-flask culture, we obtained a profile of viable and dead cells during this time.

Experimental results for the donors show live and dead cell profiles which suggest a linear HBMSC growth profile during culture. Furthermore, there is on average 10% dead cell concentration from total (dead plus viable) HBMSC.

Metabolites in medium under standard culture conditions without cells show that HBMSC consumption and production of some metabolites may be significantly overestimated. For example, glutamine concentrations decrease by 25% every day. Also, ammonia concentrations increase by 32% every day. The results suggest that under standard culture conditions HBMSC growth is accompanied by nutrient degradation and waste production that are not induced by HBMSC metabolism.

Data on metabolite degradation makes growth kinetic parameter estimation accurate. Our goal is to study how biologically significant these data are within and between donors. Thus, we are developing a numerical model to help answer these questions qualitatively and quantitatively. Based on mass balance equations on measured variables, we use Matlab[®] functions for parameter estimation and dynamic modelling.

(281) Modelling the Defective Bone Repair Bio-Scaffold Using RP-Based Tissue Engineering

Hu Q., Yang H., Lin L., Fang M.

Rapid Manufacturing Engineering Center, Shanghai University, Shangda, P. R. China

In this paper, a software modeling method is introduced to assist the fabrication of the defective bone repair bio-scaffold. An improved model structure of the bio-scaffold is established and a new defective bone repair bionic scaffold modeling method is proposed. According to the study of the biologic properties and physical properties of the bio-scaffold, the model structure is established, and the requirements of the bio-scaffold are proposed. Based on the structure and requirements, the modeling method is proposed. Image processing is used to process the medical CT images, and by 3D-reconstructing and a triangle reduction method, the optimal model of the defective bone is obtained. Then, an improved interactive hole filling method is used to get the repair model of the defective bone. And the final model of the defective bone repair bio-scaffold is obtained by a micro-structure constructing method based on Boolean operation. This method can be used in both symmetrical and unsymmetrical defective skull, which can construct a defective bone repair bio-scaffold CAD model that has the macro-shape and micro-pore. The proposed method was successfully implemented by programming based on our medical software toolkit, and the repair bio-scaffold CAD model was constructed. Through RP machine, using the polymeric blends, a nicer physical model was obtained, which meets the requirements of the bio-scaffold.

(282) Modelling Transdermal Delivery of High Molecular Weight Drugs from Microneedle Systems

Al-Qallaf B., Das D.B.

Oxford University, Dept Engineering Science, Oxford, OX1 3PG, United Kingdom

A recent approach that combines the concepts of transdermal drug delivery across the skin using patches and the hypodermic injections has been receiving significant attention in the field of the transdermal drug delivery. This involves fabrication of arrays of needles of microns in dimensions, called microneedle for transdermal drug transport. Although different microneedle designs have been fabricated, not all of them have the ability to increase the blood concentration of the drug. In many cases, designs developed based on certain criteria (e.g., strength of the microneedles) have failed to meet other criteria (e.g., permeability of skin, throughputs of the drugs, etc.). Therefore, appropriate mathematical models for drug transport for these systems, which promise to eliminate various design uncertainties, are very attractive. To address this issue and to evaluate the performance of microneedle, a mathematical framework is developed in this work. Numerical simulations are carried out to describe the pharmacokinetics of high molecular weight drugs penetrated into skin by using microneedle array. The major focus in this paper is to investigate the influences of a variety of variables related to the microneedles and their impacts on the drug transport in skin. Many relevant factors have

been examined, including the length of the microneedle, the duration of application, the size of the patch, etc. Finally, dimensional analysis has been carried out to obtain a scaling relationship between these parameters and the drug concentration in blood. The simulations have allowed identifying the significance of various factors that influence the drug delivery while designing micro-needle arrays.

Keywords: Transdermal Drug Release Systems, microneedle arrays, Computer Modelling

(283) Modification of Bioengineered Nerve Conduits with ECM Molecules

Armstrong S.J.¹, Wiberg M.², Terenghi G.¹, Kingham P.J.¹

¹Blond McIndoe Research Laboratories, University of Manchester, United Kingdom

²Department of Surgical & Perioperative Science, University Hospital, Umea, Sweden

Schwann cells (SC's) and extracellular matrix (ECM) molecules play key roles in regeneration following nerve injury. Previously we have shown poly-3-hydroxybutyrate (PHB) is a suitable biomaterial for construction of artificial nerve conduits designed to enhance nerve repair. In this study we investigated the effect of the ECM molecules, laminin, fibronectin and collagen on the attachment and proliferation of SCs on PHB and the effect that SCs have on neurite outgrowth *in vitro*. Initial attachment of SCs to PHB was not enhanced by coating the PHB with ECM molecules; approximately 60% of SCs attached after 3h for all conditions. In contrast, SC proliferation on PHB mats was enhanced by the ECM molecules, with laminin having the greatest effect.

Neurite outgrowth from NG108-15 motor neuron-like cells was determined in response to diffusible molecules released from SCs, and direct contact with SCs. The median neurite length was greatest for cells grown on laminin followed by fibronectin, and collagen. Cells grown in direct contact had significantly ($p < 0.05$ by one-way ANOVA) longer neurites than those exposed to diffusible factors alone. SCs also enhanced the percentage of NG108-15 cells extending neurites and the number of neurites per cell body in both systems.

Thus ECM molecules provide an improved substrate for SC proliferation on PHB. Incorporation of ECM molecules into nerve conduits may provide a more successful strategy for future peripheral nerve repair.

(284) Modification of Biological Scaffolds with Specific Extracellular Matrix Proteins Enhances Collagen Synthesis by Mesenchymal Stem Cells

Dreger S.A., Chester A.H., Bowles C.T., Yacoub M.H., Taylor P.M.

Imperial College London, Harefield, Middlesex, United Kingdom

Successful tissue engineering of a heart valve will rely upon the ability of the cells to remodel the supporting scaffold. The aim of this study was to assess the ability of mesenchymal stem cells (MSCs) to remodel a biological scaffold after mechanical conditioning in a bioreactor.

Porcine collagen type I/III scaffolds (Chondro-Gide®) were coated with or without human fibronectin, seeded with human bone marrow MSCs ($10^3/\text{mm}^3$) and maintained under rotary conditions for 7 days then conditioned in a bioreactor ($n = 3/\text{group}$) for a further 2 days. Cell phenotype, matrix metalloproteinases (MMP) and extracellular matrix were determined by immunocytochemistry. Cell viability was assessed using the CellTiter 96® Aqueous One Solution Cell Proliferation Assay.

MSCs attached to the scaffolds and proliferated under both rotary and mechanical conditioning in the uncoated scaffolds ($4.31 \pm 0.73 \times 10^3/\text{mm}^3$) and fibronectin coated scaffolds ($3.66 \pm 0.37 \times 10^3/\text{mm}^3$). However no significant difference was detected. Cells in both groups similarly expressed phenotype markers smooth muscle alpha-actin, vimentin, CD29 and CD44. The matrix metalloproteinases (MMP-1, -3, -8, -13, -14) were similarly expressed in both groups, however MMP-9 and HSP47 expression increased on the fibronectin coated scaffolds.

This study shows that modification of scaffold with specific ECM proteins can enhance collagen synthesis. These results have important implications for tissue engineering.

(285) Molecular Analysis of Human Osteogenesis on a Collagen-GAG Scaffold

Keogh M.B., O'Brien F.J., Daly J.S.

Royal Collage of Surgeons Ireland, Ireland

This project aims to investigate molecular osteogenesis using the human cell line hFOB 1.19 on a novel collagen-GAG porous scaffold as the basis for tissue engineered bone grafts.

In order to achieve maximal cell attachment an optimal cell seeding density curve was prepared by seeding 1.5×10^6 cells/scaffold and incubating for 48 hours. Cell viability was obtained using trypan blue exclusion method. A novel real-time viability assay called alamar blue was optimised for cells on scaffold by seeding 1.5×10^6 cells/scaffold, incubating for 48 hours. Medium was replaced with that containing 10% alamar blue for 24 hours. Cellular viability was calculated from the percentage reduction of dye.

A histological assessment of hFOB attachment and distribution within the scaffold was carried out by seeding cells onto scaffolds and culturing for ≤ 25 days. Samples then were histologically prepared prior to H&E staining. Finally, ongoing experiments examining osteogenesis with/without exposure of cells to TGF- β 1 and vitamin-D3 while seeded onto scaffold and incubating for up to 28 days have been set up.

The optimal cell seeding density was determined as 4×10^6 cells/scaffold. Alamar blue reagent can be used as a real-time viability assay for cells on scaffold. Histology results found that many pores at the centre of the scaffold contained numerous cells even after 14 days. Preliminary results using growth factors have shown an increase in viability up to 7 days. Early results demonstrate that this novel scaffold shows excellent potential as a bone graft substitute.

(286) Monocytes Stimulate Stem Cell Proliferation and Early Osteogenic Differentiation in a Co-culture System

Pirracò R.P., Marques A.P., Reis R.L.

3B's Research Group—Biomaterials, Biodegradables and Biomimetics, Dept. of Polymer Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
 IBB—Institute for Biotechnology and Bioengineering, PT Government Associated Laboratory, Braga, Portugal

The use of stem cells in tissue engineering promised to be a way to circumvent the problem of cell sourcing but it's still failing in accomplishing that. The elucidation of the effects of different types of cells over progenitor cells may lead to the discovery of new pathways to accelerate stem cell differentiation, proliferation or, ideally, both. The effect of soluble factors produced by monocytes, key players in the regenerative process, over the early osteogenic differentiation of human bone marrow stem cells was assessed in this work.

Human bone marrow cells and human peripheral blood monocytes were isolated from healthy volunteers using a differential centrifugation technique. Human stem cells were cultured in 24-well plates in a concentration of 30,000 cells/well. Cell culture inserts, with a 0.45 μm pore membrane, were seeded with 50000 monocytes/insert and placed over stem cell wells. Cells were cultured in osteogenic medium for 2, 5 and 7 days. Osteogenic differentiation and cell proliferation were assessed by quantification of Alkaline Phosphatase (ALP) and dsDNA.

Cells cultured in the presence of monocytes were found to have a higher proliferation rate in comparison to controls. Surprisingly, these cells also had higher ALP/dsDNA values from day 5 onwards, suggesting that besides the positive effect over stem cell proliferation, monocytes also stimulated their early osteogenic differentiation.

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(287) Monosize Polycationic Nanoparticles for Gene Transfer

Pişkin E., Güven G., Türkoğlu M., Kutsal T.

Hacettepe University, Chemical Engineering Department, and Bioengineering Beytepe, Ankara, Turkey

Monosized poly(styrene/poly(ethylene glycol) methacrylate/N-[3-(dimethylamino) propyl] methacrylamide [poly(St/PEG-MA/DMAPM)] cationic nanoparticles were synthesized by emulsifier-free emulsion polymerization conducted in the presence of a cationic initiator, 2,2'-azobis (2-methylpropionamidine) dihydrochloride (APDH or V-50), by using different amounts of ingredients and conditions. The nanoparticles with an average size of 71.3 and 208.2 nm (with a PDI of 1.110 and 1.031) and zeta potentials of 55.6 and 54.9 mV were used in the transfection studies. HeLa cell line and SMCs isolated from the calf-aorta were used. A green fluorescent protein expressing plasmid (pEGFP-N2) and pBLAST TIMP2 plasmid encoding TIMP-2 a 24kD protein known as metalloproteinase inhibitor (for inhibition of SMC migration) were used. Conjugates of the nanoparticles with these plasmids were used in the cell transfection studies in cell cultures. The GFP expression efficiency in HeLa cells was extremely high (up to 90%), while it was lower with primary cells (around 65–70%) which was

also very successful comparing existing non-viral vectors. TIMP2 expression was detected by Western blotting in SMC transfection with pBLAST TIMP2 plasmid.

(288) Morphological Analyses of Inkjet-Fabricated Three-Dimensional Structures

Yamaguchi K., Nakamura M., Nishiyama Y., Henmi C., Mochizuki S., Takiura K., Nakagawa H.

Tokyo Medical and Dental University, Clinical Anatomy, 1-5-45 Yushima Bunkyo Tokyo 113-8519, Japan
 Kanagawa Academy Of Science And Technology, Kawasaki-City, Kanagawa, Japan

In the manufacturing of 3D biological tissues, verification of the products itself is essential. However, the effective methods to verify such 3D structure are very limited, because 3D verification should be done for not only outer structure but also inner structure and cellular viability and distribution in the product. We have been able to construct two-colored three dimensional hydrogel structures containing various cells and materials with our original inkjet bioprinting system. In this study, we explored how to observe and evaluate the three dimensional morphology of fabricated two layered hollow tubular structures that were about 1mm in diameter and a few mm to cm in length.

We applied the computer assisted 3D construction technique that has been often used in anatomical researches to comprehend the 3D structure of the specimen. To obtain the 2D tomographic images, three approaches were applied. First was the method using slit laser light, which lights up thin sections of the structure. Second was the "Z-stack image" in which serial images were obtained according to graduated changes of the focus of microscope. Finally, we made serial frozen sections of the 3D hydrogel structure. All the sections were digitally photographed, then computer assisted 3D reconstruction was performed with a software SrfII (Ratoc, Tokyo, Japan).

With those methods, the hollow tubular structure of the hydrogel and three-dimensional distribution of the cells were observed. Computational 3D reconstruction method is useful for the morphological evaluation of the engineered 3D products.

(289) Morphological Evaluation of Potential for Differentiation in Passaged Chondrocyte Populations

Kino-oka M., Sato Y., Khoshfetrat A.B., Takezawa Y., Taya M.

Osaka Univ., Div of Chemical Engineering, Osaka 5608513, Japan

For repairing articular cartilage defects, the innovative techniques based on tissue engineering have been developed and are now entering into a practical stage of clinical application by means of grafting *in vitro* cultured tissues. One of the core processes to produce cultured cartilage is the subculture for cell expansion. However, the monolayer growth of chondrocytes often causes the dedifferentiation, resulting in immature structure of cartilage *in vivo*. Therefore, the assessment of the status for differentiation of chondrocyte cells during the cultures is required.

In the present study, the cell populations of rabbit chondrocytes at the different level of population doubling (PD) were prepared by sub-culturing in a conventional T-flask. The population was seeded

on a high-density collagen substrate and in collagen gel to evaluate the planar and spatial morphologies of passaged chondrocytes, respectively, as well as gene expression of mRNA for collagen types I and II.

The morphological estimation by cell roundness (R_c) was conducted on the collagen substrate at different PD values. Frequency of round-shaped cells with $R_c > 0.9$ decreased with increasing PD values, accompanied with the increment of mRNA level for collagen type I. The similar trend was observed in the collagen gel. Microscopic observation with image analysis revealed three-dimensional chondrocyte morphology through staining with cytoplasm which can be evaluated by spatial morphological parameter of sphericity (Sc). With an increase in PD value, frequency in spherical-shaped cells with $Sc > 0.9$ decreased, and the mRNA expression of collagen type I increased, suggesting the progress of dedifferentiation.

From these results, cell morphologies on the collagen surface and in the collagen gel are proposed to be indicators available for evaluating the potential of differentiation during cell expansion of chondrocytes, leading to a quality control of cultured cartilage.

(290) Mouse Cardiac MR Imaging of Magnetically Labeled Human Amniotic Fluid Stem Cells for Longitudinal Cell Graft Monitoring

Dawn D., Olson J., Atala A., Zhu J-M., Soker S.

Wake Forest Institute of Regenerative Medicine, Wake Forest University Health Sciences, Winston-Salem, NC, United States of America

There is a rapidly increasing interest in the use of Magnetic Resonance Imaging (MRI) for molecular and cellular imaging. We hypothesize that micrometer-sized iron particles (MPIOs) can be used for tracing stem cells for therapeutic application. We have tested high resolution MRI to track and monitor amniotic fluid derived stem (AFS) cells injected into mouse hearts.

MPIO were introduced into human AFS cells using nucleofection and were detected both by fluorescence microscopy and MRI. Flow cytometry analysis was done to quantify the number of labeled cells over several cell divisions. MPIO labeled AFS cells were injected into the heart of mice and MRI was performed at varying times after cell injection. After MRI, histology was used to confirm the location of stem cells in the heart.

Flow cytometry analysis revealed that MPIO labeled AFS cells maintain labeled for up to 14 divisions. MRI of agarose gels injected with MPIO labeled AFS cells demonstrated significant hypointense regions. Labeled AFS cells injected into mouse hearts demonstrated successful hypointense regions by MRI at 24 hours, 1, 2 weeks, 3 and 4 weeks. Results of AFS cell integration in the heart were further confirmed by histological analysis using fluorescence microscopy and Prussian blue staining.

This study represents the first report of *in vivo* MRI identification and tracking of MPIO-labeled stem cells in the mouse heart using MRI. Development of new methods for non-invasively tracking and monitoring stem cells *in vivo* will be of great importance for the future of clinical applications in cell therapy.

(291) Mouse Embryonic Stem Cell Engraftment in Healthy and Injured Mouse Lung

Lane S.¹, Rippon H.¹, Takata M.², Mahadeva R.³, Bishop A.E.¹

¹Experimental Medicine and Toxicology, Imperial College London, London, United Kingdom

²Division of Surgery, Oncology, Reproductive Biology and Anaesthetics, Imperial College London, London, United Kingdom

³Department of Medicine, University of Cambridge, Cambridge, United Kingdom

Embryonic stem cells (ESCs) are a potential source for cell therapy and tissue engineering strategies and for respiratory disease, distal, gas exchange epithelium is an obvious target. We have previously demonstrated enrichment of differentiating murine ESC cultures with mature distal airway epithelial cells (type I and II pneumocytes) and their progenitors. In this proof-of-concept study, we aimed to establish whether these cells can engraft in the lung following systemic delivery, as has been shown for bone marrow-derived cells. Differentiated, pneumocyte-enriched mESC populations were labelled using the fluorescent cell tracker CFDA SE. One million cells in 200 μ l of buffer were implanted via the tail vein into mice with an LPS-induced model of acute respiratory distress, mice with an elastase model of chronic obstructive pulmonary disease as well as healthy mice. Mice from each group were culled at 24 hours, 48 hours and 5 days after implantation. Implanted cells were traced in the lungs using the fluorescent label on U.V. light microscopy and phenotyped by immunohistochemistry with antibodies to a range of epithelium-, endothelium-, macrophage- and lung-specific markers.

Implanted cells are seen embedded in the lungs at all time points and in the largest numbers 24 hours after implantation in all mice. The frequency of labelled cell engraftment was noticeably greater in the lungs of healthy mice where they also appeared to persist longer than in injured lungs. The cells are commonly embedded in the distal epithelium of the lungs. These cells are positive for epithelial markers, and some show overlap with macrophages. As expected, the implanted mixed population contained cells with diverse phenotypes including cells of small airways and endothelial cells. As implanted cells were seen in diverse regions of the lungs it is possible to speculate that the cells embedded in locations according to their phenotypic niche. Other tissues, including the liver, showed the odd fluorescent body but too infrequently to phenotype.

Our findings support the contention that differentiated murine ESC, enriched for a specific phenotype, can colonize a specific organ. This work strongly suggests that ESC have potential in cell therapy for lung diseases.

(292) MR Surveillance of Human Tissue-Engineered Arterial Conduits in SCID Mice via SPIO Labeling

Nelson G., Roh J., Shkarin P., Fahmy T., Breuer C.K.

Yale University School of Medicine, Department of Surgery, Section of Pediatric Surgery, New Haven, CT 06520, United States of America

Background: The field of vascular tissue engineering now routinely produces functional tissue-engineered grafts via seeding vascular cells onto biodegradable polymer constructs. However, the mechanisms by which tissue-engineered grafts develop *in vivo* are not well understood. In this study, we propose and validate the use of superparamagnetic iron oxide (SPIO), a T2 negative-contrast agent, to label vascular cells and then noninvasively monitor their

contribution to an implanted tissue-engineered arterial conduit (TEAC) *in vivo* via MRI.

Methods: Human aortic smooth muscle cells (hAoSMCs) were labeled with SPIO nanoparticles (Combidex) coated with poly-L-lysine. Labeled hAoSMCs were then seeded onto a biodegradable polymer scaffold composed of a poly-L-lactate mesh cylinder coated with a 50:50 copolymer of poly[L-lactide-co- ϵ -caprolactone]. After six days of *in vitro* culture, human aortic endothelial cells (hAoECs) were seeded within the graft lumen. Our TEACs were implanted into SCID beige mice as an aortic interposition graft and the mice were imaged at post-operative days 4, 11, and 18 to determine the relative ease of locating SPIO-labeled grafts *in vivo*.

Results: At each time-point, T2-weighted gradient and spin echo MR sequences allowed easy identification of labeled hAoSMCs attached to the scaffold within the murine abdomen. Post-explant histology from each time-point has revealed the co-localization of smooth muscle actin filaments and Prussian blue positive iron aggregates, verifying that SPIO-labeled hAoSMCs continue to contribute to the wall of our TEACs up to 3 weeks post-implantation. These preliminary data validate our MR results and establish the merit of this technology for noninvasive *in vivo* TEAC surveillance.

(293) Muscle Derived Stem Cell Autografts in a Rat Model of Fecal Incontinence

Kang S.B.¹, Lee H.N.², Lee J.Y.², Park J.S.³, Lee J.Y.²

¹Department of Surgery, Seoul National University College of Medicine, Korea

²Department of Urology, College of Medicine, Catholic University of Korea, Holy Family Hospital, Korea

³Department of Surgery, Chung-Ang University College of Medicine, Seoul, Korea

Background: Muscle-derived stem cells (MDSC), which we obtained *in vitro*, were effective for improving urinary function in a rat urinary incontinence model. To date, there have not been any studies about MDSC injection into the anal sphincter in a fecal incontinence model.

Purpose: We aimed to determine whether injecting MDSC can improve the functional properties in a fecal incontinence model in rats.

Methods: We established the fecal incontinence model by cryo-injury of liquid nitrogen in the anal sphincters of normal female Sprague-Dawley (S-D) rats (3 week-old, 250–300 g). Cells isolated from the gastrocnemius muscle of normal female S-D rats were used for purification of the MDSC using the preplate technique. Normal female S-D rats were divided into three groups ($n = 5$ for each group); a normal control group; a cryo-injured group; MDSCs (3×10^6 cells) injection group in a cryo-injured rat. All groups subsequently underwent contractility experiments using strips of anal sphincter one week later.

Results: Contraction of the anal sphincter strip in the cryo-injured group was lower significantly than in the normal control group after acetylcholine (10^{-4} mol/l) and KCL (10^{-4} mol/l) treatment

(17.3 ± 3.4 vs 35.1 ± 6.2 g per gram tissue, $p = 0.001$; 18.4 ± 7.9 vs 46.9 ± 14.1 g per gram tissue, $p = 0.006$). In the MDSC injection group, contraction of the sphincter strip was higher than in the cryo-injured group after acetylcholine (10^{-4} mol/l) and KCL (10^{-4} mol/l) treatment, but not significantly different (20.5 ± 21.3 vs 17.3 ± 3.4 g per gram tissue, $p = 0.748$; 31.0 ± 14.2 vs 18.4 ± 7.9 g per gram tissue, $p = 0.120$).

Conclusion: Autologous MDSC graft may be a tool for improving anal sphincter function, although it did not effect significant improvement in a rat model of fecal incontinence. Further studies are required in a larger group for long-term follow up.

(294) Muscle Derived Stem Cells Differentiate into Liver-Like Cells

Vos C.¹, Pan H.¹, Alston S.¹, Zheng B.¹, Huard J.^{1,2,3}, Li Y.^{1,2,4}

¹Stem Cell Research Center (SCRC), Children's Hospital of Pittsburgh, ²Department of Orthopaedic Surgery, ³Department of Molecular Genetics and Biochemistry, and ⁴Department of Pathology, University of Pittsburgh, United States of America

The liver is an important organ in the human body capable of regeneration after injury. However, the liver often fails to repair itself if it is seriously damaged or diseased. Stem cells could have the ability to help repair injured or diseased liver tissue. Post-natal stem cells, such as muscle-derived stem cells (MDSCs), can differentiate to form muscle, blood vessel, bone, cartilage, and other types of cells. The aim of this project is to investigate whether MDSCs can differentiate into hepatocytes *in vitro*, and repair traumatically injured liver *in vivo*. In this study, we selected MDSCs co-cultured with a hepatocyte cell line-HepG2 to test whether the stem cells could differentiate into liver-like cells based on the expression of hepatocyte marker proteins: Cytokeratin (CK)-19, CK-18, hepatocyte and albumin. We found some MDSCs began to express CK-18 and/or CK19, and albumin after the co-culture within 48 hours. In a hepatomatory mice model, we discovered that MDSCs stay and began to express some hepatic markers, and may differentiate and/or fuse into liver cells after systemic delivery. The results from this experiment show that MDSCs have the capacity to differentiate into liver-like cells *in vitro* and *in vivo*, providing valuable information that can be used in further investigations involving MDSCs and liver repair. Results from this experiment are highly significant from a cell therapy standpoint, because they could serve as the basis for further clinical applications aimed toward the treatment of injured or diseased liver tissue.

(295) Muscle Progenitor Cells for the Restoration of Irreversibly Damaged Sphincter Function: A Pre-Clinical Study

Eberli D., Yoo J., Soker S., Atala A.

Institute for Regenerative Medicine, Wake Forest University School of Medicine and University of Zurich, Departement of Urology, United States of America

Background: Multiple treatment modalities, including surgeries and injection therapies, have been tried for urinary incontinence. However, none of these methods is able to entirely restore normal

sphincter muscle function. We explored the possibility of achieving functional recovery of the urinary sphincter muscle using cell-based therapy. We investigated whether irreversibly damaged urinary sphincter function could be fully restored using autologous muscle progenitor cells (MPC).

Methods: A model of urinary sphincter insufficiency was created by excising approximately 80% of the sphincter muscle in 26 dogs. Sphincter function was assessed by urodynamic studies on normal and damaged sphincters. Autologous MPC were injected into the damaged sphincter muscle and the animals were followed for up to 6 months after injection.

Results: Animals with MPC injection were able to recover the sphincter pressure to approximately 80% of normal, while the pressures in the control animals dropped and remained at 20%. Histologically, the implanted cells that were labeled with the fluorescent dye tracer survived and formed new innervated muscle fibers.

Conclusion: Autologous muscle progenitor cells are able to restore otherwise irreversibly damaged sphincter function in dogs. The injected cells are able to survive and form mature tissue within the damaged sphincter. This study demonstrates the feasibility of using autologous muscle precursor cells for the functional restoration of urinary sphincter muscle in patients with sphincter insufficiency.

(296) Muscle-Nerve Interactions in an *In Vitro* 3D Collagen Gel Model

Hakim W., Solomon A., Salih V., Mudera V., Lewis M.P.

UCL Eastman Dental Institute and Institute of Orthopaedics and Musculoskeletal Sciences, 256 Grays Inn Road, London, WC1X 8LD, United Kingdom

Background: The potential applications of a functional 3-D neuromuscular construct (NMC) include its use as a 3-D model for developmental experiments—as an interim between monolayer culture and *in vivo* testing and maybe even replacing it in some instances—and extend to future applications in skeletal muscle tissue replacement.

Method: 3D collagen constructs (75×25×15 mm) were seeded with MPC (adult human primary muscle precursor cells), DRG's (chick embryos (E7-10)) or a combination of both. The constructs were held at either end, developing an endogenous static load causing cell alignment parallel to lines of principal strain developed. This promoted formation of syncytial myotubes. Gene expression profiles of myogenin and nAChR-epsilon were quantitated.

Results: MPC seeded constructs showed evidence of predictable syncytial myotubes. Addition of DRG's and co-culturing demonstrated cell viability and alignment over the testing period. Gene expression showed an overall increase in both myogenin (0.26 ± 0.03 for the co-cultures vs. 0.08 ± 0.01 for MPC alone) and nAChR-epsilon (0.85 ± 0.26 for the co-cultures vs. 0.41 ± 0.11 for MPC alone) mRNA expression when compared to single culture only. This is an indication of synergistic interaction between the two cell types.

Conclusion: A 3-D co-culture system has been created where the nerve-component is cellular as opposed to explanted. Some form of muscle-nerve interaction has been demonstrated as average myogenin mRNA levels are increased in co-cultures. This implies that the nerve cells are having some effect on myoblast differentiation. In addition, nAChR-epsilon expression increase is indicative of nascent muscle-nerve interfaces forming.

(297) Myogenic Differentiation of Bone Marrow Stem Cells in Response to Novel PLLA-Collagen Scaffolds

Pu F.R.¹, Rhodes N.P.¹, Lefranc O.², Codoro C.², Bayon Y.², Gravagna P.², Thérin M.², Hunt J.A.¹

¹UKCTE, Clinical Engineering, University of Liverpool, Liverpool, United Kingdom

²Sofradim Production, 116 Avenue De Formans, 01600 Trevoux, France

The response of bone marrow derived SMCs on novel collagen-PLLA scaffolds for bladder tissue engineering was investigated; these cells were compared to SMCs isolated from porcine bladder. Phenotypic markers and extracellular matrix proteins were evaluated at both the transcriptional level using real-time RT-PCR and the protein level by western blotting.

3-D composite sponges comprised of PLLA textile combined with formulations of cross-linked porcine collagen type I were investigated. The collagen density and the extent of collagen reticulation (High GTA or Low GTA) were varied to produce potentially significant differences in the sponges. MSCs were isolated from porcine bone marrow and SMCs isolated from fresh porcine bladder muscle. MSCs were cultured on scaffolds *in vitro* for 1, 7 and 28 days in myogenic medium. The gene expression of -SMA, calponin, collagen I, collagen III and GAPDH was studied by real-time RT-PCR. The production of -SMA protein was determined by immunohistochemistry and western blotting.

The greatest number of cells grew on the Low GTA small pore scaffold for both SMCs and MSCs. Increased gene expression of -SMA was also observed on the Low GTA material for both SMCs and MSCs. It was demonstrated that SMCs and MSCs adhered and proliferated whilst maintaining a smooth muscle cell phenotype and that MSCs were capable of expressing SMC markers while in the myogenic medium. It was concluded that a small pore, Low GTA scaffold was an excellent candidate for the culture of urological tissue.

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(298) Nanoengineered Polymeric Capsules for Cancer Therapy

Erokhina S.¹, Pastorino L.², Caneva Soumetz F.², Ruggiero C.²

¹University of Parma, Department of Physics, Viale Usberti 7 A, Parma, 43100, Italy

²University of Genoa, Department of Communication, Computer and System Sciences, Via Opera Pia 13, Genoa, 16145, Italy

Paclitaxel is one of the anticancer agents most often used in clinical oncology practice for the treatment of ovarian, breast and non-small cell lung cancers [1]. Effective utilization of this agent

demands its encapsulation with successive directed release in the appropriate zones. Nanoengineered polymeric capsule (NPC) is a new and very effective tool for the encapsulation and smart release of different compounds [2].

The capsules were fabricated in the following way. PSS-PAH multilayer structure was formed by polyelectrolyte self-assembling (layer-by-layer technique) on the surface of 5.0 µm templates of CaCO₃. Then, the templates were dissolved by the variation of the solvent composition and the hollow capsules were formed. Paclitaxel penetrate into the capsule when pores in their shell were opened at lower pH value (less than 6.0) with successive encapsulation rising the pH above 7.5. Such NPCs were used for paclitaxel release in the media with slight acid properties. Encapsulation and release of the compound was studied by optical absorbance and Raman spectroscopies.

References

1. C. Joyce. (1993). Taxol: search for a cancer drug. *Bioscience* 43. p.133–136.
2. G.B. Sukhorukov, M. Brumen, E. Donath, and H. Mohwald. (1999). Hollow Polyelectrolyte Shells: Exclusion of Polymers and Donnan Equilibrium. *J. Phys. Chem. B*. 103. pp. 6434–6440.

(299) New Therapy for Intractable Otitis Media: Regeneration of the Mastoid Air Cells

Kanemaru S., Yamashita M., Umeda H., Ito J.

Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Japan

Introduction: Poor development of the mastoid air cells in the temporal bone is a common anatomical feature of chronic otitis media. Mastoid air cells have a gas exchange function. Once this function failed, intractable otitis media would be prolonged. Therefore, the aim of this study is to regenerate functionally the mastoid air cells by in situ tissue engineering.

Materials and Methods: Hydroxyapatite (HA) of honeycomb-like structure was used as artificial pneumatic bones. It has a high ratio (90%) of macro pore and is coated with collagen. 26 patients with cholesteatoma, adhesive otitis media, and purulent chronic otitis media undertook this new operation. At the first stage of tympanoplasty, HA was implanted into the newly opened mastoid cavity. CT scan was then performed to determine whether the mastoid air cells were regenerated or not. At the second stage of operation, histopathological examinations and measurement of middle ear pressure were performed.

Results: Aeration in the mastoid cavity was observed in 21 of the 26 patients 1 year after the second operation. Moreover, the pneumatic structure in mastoid cavity was regenerated in 18 patients. In these successful cases, the surface of the implanted HA was covered by mucosa with newly formed capillaries. The middle ear pressure regulation was recovered.

Conclusion: We succeeded in regeneration of the mastoid air cells through the implantation of collagen coated HA in patients with intractable otitis media. In situ tissue engineering method is a possible new treatment for intractable otitis media.

(300) Non-invasive Monitoring of Tendon Matrix Structure

Cambrey A.D., Seumenicht O., Birch H.L., Brown R.A.

University College London, Tissue Repair and Engineering Centre, Institute of Orthopaedics, London HA7 4LP, United Kingdom

We have established that elastic scattering spectroscopy (ESS) could be an effective non-invasive monitoring technique to demonstrate collagen fibril alignment during loading (1). In this present study we aim to establish that ESS can be used to quantitatively characterise changes in collagen fibre architecture in degenerative tendon, with relevance to monitoring engineered implants following reconstructive surgery. Equine superficial digital flexor tendons with gross disruptive lesions and swelling in the mid-metacarpal region were compared to normal tendons. A fibre optic probe delivered pulses of light (320–860 nm) to the tendon surface, the spectra of backscattered light were collected, and the optical anisotropy factor (AF) from the backscatter intensity ratio (500 nm) in orthogonal planes was determined. Variations in AF were compared with tendon changes demonstrated by histology and ultrasonography. AF at the lesion sites was almost 4 times lower than that determined in normal tendon ($p < 0.05$), indicating substantial matrix disorganisation. This close anatomical correlation was confirmed by histology and as a hypoechoic region by ultrasonography. Indeed, altered AF was also detectable distal to overt lesion sites. Our results suggest that spatial anisotropy as detected by ESS can be used for minimally invasive, quantitative monitoring of early degenerative and reparative changes to tendon matrix structure, and may also be useful in monitoring engineered connective tissues post-implantation.

Reference

1. Kostyuk O, Birch HL, Mudera V, Brown RA. Structural changes in loaded equine tendons can be monitored by a novel spectroscopic technique. *J Physiol*. 2004;554:791–801.

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(301) Novel Biodegradable Cryogels Made of

Eğri S.¹, Vargel İ.², Korkusuz P.³, Plieva F.⁴, Galaev, I.⁴, Pişkin E.¹

¹Hacettepe University, Chemical Engineering Department and Bioengineering Division, Beytepe, 06800 Ankara, Turkey

²Kırıkkale University, Faculty of Medicine, Plastic and Reconstructive Surgery Division, Kırıkkale, Turkey

³Hacettepe University, Faculty of Medicine, Department of Histology and Embryology, Ankara, Turkey

⁴Lund University, Department of Biotechnology, P.O. Box 124, S-221 00 Lund, Sweden

In this study we first synthesized a series of co- and ter-polymers of L-lactide and ε-caprolactone with polyethylene glycol (PEG) with different molecular weights in the presence of Sn(Oct)₂ as catalyst, by changing the molecular weight of PEG (3000, 8000, 10000). ¹H-NMR results confirmed the co- and terpolymer formation. DSC analysis showed that incorporation of PEG lowers the melting temperatures depending on both the amount and molecular weight of PEG incorporated. The number and weight average molecular weights, and polydispersity indices obtained by GPC using PS standards were 18–40 kDa, 44–103 kDa, and 1.8–2.9, respectively.

Macroporous scaffolds with highly open and interconnected pore morphologies were obtained by applying a cryogelation technique. These scaffolds are soft and flexible and exhibit very high water uptakes (up to 400% based on dried initial weights) and very rapid swelling rates in aqueous medium (only a few seconds to reach fully swollen phase). Mechanical properties, swellabilities, and degradation behaviors in aqueous media were found primarily dependent on molecular weight and composition. Selected ones were implanted into different tissues of the model animals (rats), namely in critical size cranial bone defects (inlay), iliac bone defects (onlay), in ear-cartilage, and subcutaneously, to obtain preliminary tissue responses. Histological evaluations exhibited that the scaffold revealed a mild to moderate inflammatory reaction diminishing with time, and changing significantly with the implantation area.

(302) Novel Bottom Up Approach to Realize Bioactive Scaffold by Biodegradable Microspheres

Luciani A., Coccoli V., Orsi S., Guarino V., Causa F., Ambrosio L., Netti P.A.

Dept. Materials and Production Engineering and CRIB, University of Naples "Federico II," P.le Tecchio 80, 80125, Naples, Italy

One of the most important challenges in tissue engineering is the design of suitable porous biodegradable scaffold. Ideally, a scaffold should have the following characteristics: three-dimensional and highly porous with an interconnected pore network, biocompatible and bioresorbable with a controllable degradation and resorption rate to match tissue growth *in vitro* and/or *in vivo* as well as mechanical properties to match those of the tissues at the site of implantation [1]. Moreover, it has also been reported that an active scaffold for tissue engineering should enhance and guide the process regeneration through the sequestration and delivery of specific bioactive factors [1]. This study presents a novel bottom up approach to realize bioactive scaffold by biodegradable microspheres sintering. A double-emulsion process was used to fabricate microspheres made of poly-ε-caprolactone (PCL) with encapsulated Bovine Serum Albumine (BSA), which served as a model drug. Three-dimensional porous matrix for bone repair applications was obtained through thermal and mechanical microspheres compaction. Pore size, degree of porosity and interconnectivity were controlled by microspheres dimension and processability. It was shown that the matrix provided a total interconnected porous structure with low degree of porosity, while maintaining the necessary biomechanical support. Moreover, by sintering protein loaded microspheres, bioactive scaffold capable of sequestration and delivery of biological agent had been obtained.

Reference

1. D.W.Hutmacher(2000)–Scaffold in tissue engineering bone and cartilage–Biomaterial 21 p. 2529–2543.

(303) Novel Cell Seeding Method for the Tissue-derived Acellular Scaffolds

Ehashi T., Somekawa S., Udagawa H., Fujisato T.

National Cardiovascular Center Research Institute, Dept Regenerative Medicine & Tissue Engineering, Osaka, Japan

For purpose to repair soft tissues, many research groups have been using porous scaffolds incorporated with cells *in vitro* or *in vivo*. General way for seeding cells in porous scaffold is dropping cell suspension on it, and then the cells may impenetrate into the scaffold spontaneously. However, it is not easy to seed cells completely inside of the scaffold having small pores. In this study, non-needle injector was applied to cell seeding into the tissue-derived acellular scaffolds.

The acellular cardiac muscle scaffolds were prepared by cold isostatic ultra-high pressure treatment (980 MPa for 10 min. at 4 degree Celsius) following washing steps. Cultured L929 cells were harvested from the culture dish and suspended in the PBS(-) at the density of 1.0×10^6 cells/mL. They were then injected into the acellular scaffold with the non-needle injector for percutaneous insulin administration (SHIMAJet®; Shimadzu Corporation, Japan) or the conventional syringe.

From the histological study, all nuclei were washed out from the scaffold and small pores in the range of 20–50 μm were observed among the cell skeletons. Most of the cells seeded into the scaffold with the injector were stained by calcein-AM as viable cells 24 hrs after the injection. They were scattered over a wide area in the scaffold, whereas the cells were located in cellular aggregation when injected by the conventional syringe. In conclusion, the non-needle injector may be suitable for the cell seeding into the small pore scaffolds.

(304) Novel Chitosan/Poly (Butylene Succinate) Scaffolds for Cartilage Tissue Engineering

Alves da Silva M., Crawford A., Mundy J., Correlo V.M., Sol P., Battacharya M., Hatton P.V., Reis R.L., Neves N.M.

3B's Research Group, Department of Polymer Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Conventional treatments for articular cartilage defects like prosthetic surgeries have considerable limitations, especially the frequent need for revision. Tissue engineering has emerged as a potential solution for this problem, aiming at regenerating the lost or damaged tissue. This promising therapeutic route, despite the scientific efforts to repair articular cartilage loss, has not led to a complete regeneration of the functional tissue. The present work aims at evaluating the potential of 50% chitosan, 50% PBS scaffolds for a cartilage tissue engineering approach, using primary bovine articular chondrocytes.

Chondrocytes from bovine articular cartilage were isolated, according to standard procedures. Cells were seeded at a density of 6.5×10^6 cells/scaffold on sterile scaffolds of CPBS 50/50 using spinner flasks. After 72 hours, the hybrid constructs were changed into Petri dishes and cultured under rotational agitation for up to 4 weeks. Constructs were characterized by scanning electron microscopy, histological and immunological analysis and real time PCR.

Cells colonized the entire scaffold and were able to produce extracellular matrix. Immunolocalisation of collagens type I and II confirmed the presence of these proteins. Normalized Expression Ratio of collagen types I and II was higher in dynamic conditions when compared with control conditions (static culture). Data obtained support the conclusion that the newly developed CPBS scaffolds are very promising for cartilage tissue engineering applications.

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(305) Novel Glasses to Engineer Human Craniofacial Muscle

Shah, R., Hunt, N.P., Mudera, V., Knowles, J.C., Lewis, M.P.

UCL Eastman Dental Institute, 256 Gray's Inn Road, London WC1X 8LD, United Kingdom

Human muscle precursor cells (hMPCs) may be extracted using well-established explant techniques and allowed to undergo differentiation to form prototypic muscle fibres *in vitro* (1). Novel degradable phosphate-based glass fibres can support the proliferation and differentiation of craniofacial hMPCs and have the potential for use in tissue engineering applications (2). Glass composition dictates degradation rate and the original glasses had a low rate of solubility. Further investigation into human muscle engineering has involved glasses with a higher solubility rate used in isolation and as a composite encased within collagen.

hMPCs were seeded at $1 \times 10^6 \text{ ml}^{-1}$ on collagen-coated glass fibres, glass fibres within collagen and within 3D collagen alone. The seeded scaffolds were maintained at 37°C in a humidified atmosphere of 5% CO₂ in air for 17 days. Modulation contrast microscopy and RT-PCR was used to determine outcome.

By day 17, hMPCs cultured on glass fibre scaffolds had attached, proliferated and differentiated to form organised prototypic muscle fibres. In comparison, hMPCs cultured in 3D collagen scaffolds and within composite glass fibre/collagen constructs showed a random disorganised arrangement.

Artificial phosphate-based glass fibres have been produced to form a biomimetic scaffold. The parallel nature of the fibres has encouraged hMPCs to form organised muscle fibre-like structures along the length.

References

1. Lewis M.P. et al. (2000) Gelatinase-B (matrix metalloproteinase-9; MMP-9) secretion is involved in the migratory phase of human and murine muscle cell cultures. *J Mus Res Cell Motil* 21: 223–233.
2. Shah R. et al. (2005) Craniofacial Muscle Engineering Using a 3-Dimensional Glass Construct. *Biomaterials* 13: 1497–1505.

(306) Novel Nano-Composite Biomaterial for Osteochondral Tissue Engineering

Marcacci M., Kon E., Zaffagnini S., Delcogliano M., Filardo G., Giardini R., Fini M., Pressato D., Arcangeli E., Quarto R.

Rizzoli Orthopaedic Institute, Via di Barbiano 1/10, Bologna 40135, Italy

Introduction: Osteochondral articular defects represent a key concern in orthopaedic surgery. The objective of this randomized controlled animal study was to test performance of a newly developed type-I collagen-hydroxyapatite (HA) nanostructural bio-mimetic osteochondral (O.C.) scaffold which reproduces cartilage-subchondral bone morphology.

Methods: A gradient composite O.C. scaffold, based on type-I collagen-HA, was obtained by nucleating collagen fibrils with hydroxyapatite nanoparticles at physiological conditions.

After medial arthrotomy of right hind-paw and condyle exposure, a bi-lateral osteochondral lesion, 7 mm diameter and 10 mm deep, was induced in 20 sheep until bleeding appeared. Animals were assigned to five treatment groups: scaffold alone, scaffold loaded with autologous platelet-rich plasma, scaffold cultured *in-vitro* with autologous chondrocytes, scaffold loaded with autologous (freshly-digested) chondrocyte suspension and empty defect (control). Six months after surgery, animals were evaluated for gross observations, histology and radiographic images for osteo-integration.

Results: At 6 months of follow up, gross evaluation and histology of specimens exhibited good integration of the chondral surface and the hyaline-like tissue regeneration for all groups except for empty control group. Significantly better bone regeneration was observed in the group with the scaffold alone and the scaffold loaded with autologous chondrocytes. Incomplete bone defect filling and irregularity of bone-cartilage surface was detected in the group where PRP was added to the scaffold. No bone and cartilage defect healing occurred in the control group where the defect was filled with fibrous tissue. Microradiography images showed improvement in subchondral bone healing when compared to the control. No bone growth into the chondral layer was observed for all groups.

Conclusions: The results of the present study demonstrated that this novel O.C. scaffold may act as a suitable matrix facilitating regeneration of hyaline-like cartilage.

(307) Novel Tissue Engineering 3D Scaffolds for Spinal Cord Injury Based on Starch/Polycaprolactone Blends: Development and Preliminary Assessment of Their Biological Performance

Salgado A.J.¹, Sousa R.A.^{2,3}, Oliveira J.T.^{2,3}, Silva N.^{2,3}, Neves N.M.^{2,3}, Reis R.L.^{2,3}, Sousa N.¹

¹Life and Health Science Research Institute, School of Health Sciences, University of Minho, Braga, Portugal

²3B's Research Group—Biomaterials, Biodegradable and Biomimetics, Department of Polymer Engineering, University of Minho, Braga, Portugal

³IBB, Institute of Bioengineering and Biotechnology, PT Associated Lab, Braga, Portugal

With the advent of Tissue Engineering and Regenerative Medicine concepts, innovative methodologies could be envisaged for Spinal Cord Injury (SCI) regeneration, namely through the combination of 3D scaffold with an appropriate source of cells and growth factors. In this sense the objective of the present work was to determine the effects of a blend of starch with polycaprolactone (SPCL) aimed to be used in SCI repair, on the viability and proliferation of central nervous systems derived cells, such as neurons and glial cells, and further develop a dual phase 3D scaffold aimed at SCI regeneration based on these materials. For this purpose hippocampal neurons and glial cells were plated on SPCL filaments, previously deposited on polystyrene coverslips at intervals of 1–2 mm. Light and fluorescence microscopy observations revealed that both cell populations were not affected by the presence of the SPCL based biomaterial. On the contrary total protein

quantification and MTS test indicated that both cell viability and proliferation rates were similar to those obtained for the control. Furthermore it was also observed that both neuronal and glial cell populations could adhere to the SPCL filaments surface, suggesting that the surface properties of latter might be amenable for CNS cells adhesion and proliferation. Moreover the SPCL based biomaterials did not seem to elicit an active response by the inflammatory mediators of the CNS, microglial cells, as the latter were not adhered to the biomaterial and did not disclose drastic changes on their morphology. 3D scaffolds were then produced by using a combination of 3D plotting, a rapid prototyping technology, and thermal conformation. Characterization techniques reveal that the latter possess mechanical properties adequate for the above referred goals, disclosing at the same time a non-cytotoxic behaviour. Consequently it is believed that SPCL based blends might be suitable for future studies on SCI regenerative medicine.

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(308) Off-the-Shelf Hybrid Constructs for Bone Tissue Engineering

Geuze R.E., Alblas J., Kruijt M.C., Verbout A.J., Dhert W.J.A.

University Medical Center Utrecht, Dept of Orthopaedics, Heidelberglaan 100, PO BOX 85500, 3508 GA Utrecht, The Netherlands

Construction of bone grafts for regenerative medicine would highly benefit from off-the-shelf components, such as allogeneic cells and blood-borne growth factors from platelet concentrate. Although allogeneic mesenchymal stem cells (MSCs) are considered immunosuppressive, their use in transplantation studies is still cautioned. In this study we used allogeneic MSCs and compared them with per-operatively aspirated bone marrow (BM) and platelet gel (PG), being off-the-shelf products. Ten goats received six different hybrid constructs consisting of BCP scaffolds impregnated with either PG or plasma and after that seeded with BM, allogeneic MSCs or left empty. All constructs were implanted in the paraspinal muscles for nine weeks, with fluorophores administered at two, three and five weeks, to assess onset of bone formation. Analysis of the explanted samples, by basic fuchsin/methylene blue staining of MMA-embedded sections, revealed that allogeneic MSC seeding on scaffolds had a statistically significant positive effect on bone formation compared to the non-cell seeded scaffolds (ANOVA according to a randomized block design and Bonferroni corrected post-hoc test). Fresh bone marrow did not enhance ectopic bone formation. The PG, which contained clinically relevant amounts of growth factors, also did not result in more bone. Fluorophore incorporation results indicate that the presence of seeded cells in the constructs accelerates bone formation. We conclude that a hybrid construct containing allogeneic MSCs is a suitable off-the-shelf product for bone tissue engineering.

(309) Optimisation of Bioglass® Based Scaffolds for Bone Tissue Engineering

Bretcanu O.¹, Chen Q.¹, Misra S.K.¹, Roy I.¹, Verne E.², Brovarone C.V.¹, Boccaccini A.R.¹

¹Department of Materials, Imperial College London, Prince Consort Road, London, SW7 2BP, United Kingdom

²Department of Molecular and Applied Biosciences, University of Westminster, London W1W 6UW, United Kingdom

³Department of Materials Science and Chemical Engineering, Politecnico di Torino, 10129 Turin, Italy

Bioglass® is a well-known bioactive material which has been used as bone replacement for more than 15 years. More recently, Bioglass® has started to be considered for the development of highly porous scaffolds for bone engineering. In one successful development, our group has patented a method to produce Bioglass® based glass-ceramic scaffolds of tailored bioactivity and degradation behaviour by using the foam replication method. This method consists in impregnating a polyurethane (PU) foam with Bioglass® slurry followed by a heat treatment (at 1100°C). During heating, the PU burns out and the glass sinters and partially crystallises, leaving a well defined porous glass-ceramic structure.

In order to increase the scaffolds mechanical properties and structural integrity, the scaffolds were optimised in this project by coating the foam struts with a thin biodegradable polymer layer. Two different biodegradable polymeric coatings were tested: poly(D,L-lactic acid) and poly(3 hydroxybutyrate). Another approach followed to optimise the performance of the scaffolds is the fabrication of scaffolds with a graded pore structure which mimics natural bone architecture. In this case, different types of Bioglass®-derived scaffolds with continuous or stepwise gradient of porosity were produced. The starting materials were preformed PU foams, obtained by compressing them in specific aluminium foil moulds at low temperatures (200°C).

The new scaffolds were characterised by scanning electron microscopy, X-ray diffraction analysis and mechanical property tests. The bioactivity was investigated using simulated body fluid. The application of the optimised scaffolds in bone and osteochondral tissue engineering will be discussed.

(310) Optimisation of Cell Seeding Techniques for Improved Cell Attachment, Proliferation and Migration in Scaffolds for Tissue Engineering

Murphy C.^{1,2}, Lee L.W.¹, Farrell E.^{1,2}, O'Brien F.J.^{1,2}

¹Department of Anatomy, Royal College of Surgeons in Ireland, Dublin, Ireland

²Trinity Centre for Bioengineering, Trinity College, Dublin, Ireland

One of the main constraints in the development of engineered tissue has been a lack of nutrient delivery to and waste removal from the centre of implanted constructs, often resulting in avascular necrosis (1). This study aimed to optimise seeding techniques to improve cellular migration into the centre of a collagen-glycosaminoglycan (CG) scaffold prior to proliferation and production of extracellular matrix.

CG scaffolds were fabricated using a freeze-drying method described previously (2) and then seeded with MC3T3-E1 osteoblastic cells using 3 different techniques: (i) both scaffold sides were seeded, (ii) only one side seeded, (iii) seeded by syringe injection. A second group involved a dynamic environment whereby the 3 groups above were placed on a dynamic rocker for 30 min post seeding.

Scaffolds were assessed 24 h, 48 h and 7 d post-seeding for cellular metabolic activity (AlamarBlue™), cell density (Hoechst 33258).

Cell viability was maintained over the 7 day culture period on all scaffolds. However both DNA and metabolic assays demonstrate dynamic seeding, using both standard and syringe method, to have highest levels of cell attachment and proliferation. However cell distribution within the scaffold will be assessed using Haematoxylin and Eosin staining.

Current work involves seeding with mesenchymal stem cells (MSCs) to determine whether the optimal seeding technique is cell type dependent.

References

1. Burg K. J. L., (et al), J Biomed Mater Res 2000; 51: 642–649.
2. O'Brien F.J., (et al), Biomaterials 2005; 26: 433–41.

Acknowledgements: SFI & Integra.

(311) Optimised Dynamic Conditioning Enhances *In Vitro* Development of Autologous Fibrin-Based Tissue Engineered Heart Valves

Flanagan T.C.¹, Cornelissen C.¹, Koch S.¹, Tschöke B.¹, Sachweh J.S.², Schmitz-Rode T.¹, Jockenhoevel S.¹

¹Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, Pauwelsstrasse 20, 52074 Aachen, Germany

²Department of Paediatric Cardiac Surgery, University Hospital, Pauwelsstrasse 30, 52074 Aachen, Germany

Our group has previously demonstrated the synthesis of a completely autologous fibrin-based heart valve structure. The present approach aims to guide more mature tissue development in fibrin-based valves based on *in vitro* conditioning in a custom-designed bioreactor system.

Moulded fibrin-based tissue-engineered heart valves seeded with ovine carotid artery-derived cells were subjected to 12 days of mechanical conditioning in a bioreactor system. The bioreactor pulse rate was increased from 5 to 10 b.p.m. after 6 days, while a pressure difference of 20 mm H₂O was maintained over the valve leaflets. Control valves were cultured under stirred conditions in a beaker. Cell phenotype and extracellular matrix (ECM) composition were analysed in all samples and compared to native ovine aortic valve tissue using routine histological and immunohistochemical techniques.

Conditioned valve leaflets showed reduced tissue shrinkage compared to stirred controls. Limited ECM synthesis was evident in stirred controls, while the majority of cells were detached from the fibrin scaffold. Dynamic conditioning increased cell attachment/alignment and expression of α -smooth muscle actin, while enhancing the deposition of ECM proteins, in particular types I and III collagen.

The present study demonstrates that the application of low pressure conditions and increasing pulsatile flow not only enhances seeded cell attachment and alignment within fibrin-based heart valves, but dramatically changes the manner in which these cells generate ECM proteins and remodel the valve matrix. Optimised dynamic conditioning, therefore, might accelerate the maturation of surgically feasible and implantable autologous fibrin-based tissue-engineered heart valves.

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(312) Optimization of High-quality Total RNA Isolation from Different Cartilaginous Tissues

Huang C.L., Vonk L.A., Kroeze R.J., Lu Z.F., Helder M.N., Bank R.A., Wuisman P.I.J.M., Zandieh Doulabi B.

Department Orthopaedic Surgery, Vrije University Medical Center (VUMC), Amsterdam, The Netherlands

Department of Oral Cell Biology, Academic Center For Dentistry Amsterdam (ACTA), Amsterdam, The Netherlands

Numerous studies aim at bypassing the intrinsic inability of cartilage to spontaneously heal itself. However, studies to understand the molecular mechanisms of degeneration and regeneration have been seriously hampered because of problematic isolations of RNA from cartilaginous tissues by the combination of their low cell content and proteoglycan-rich extracellular matrices. The objective of this study was to compare and optimize RNA extraction procedures for different cartilaginous tissues, i.e. articular cartilage (AC), meniscus (M), and intervertebral disc nucleus pulposus (NP) tissue.

Tissue samples were collected from goat, separated into two parts each, and homogenized by MagNA Lyser (Roche) or Freezer Mill (Spex CertiPrep), respectively. Each homogenate was subsequently divided over four groups, and subjected to RNA isolation using Trizol (Invitrogen), RNeasy Lipid kit (Qiagen), RNeasy Fibrous kit (Qiagen) and Aurum Total RNA Fatty and Fibrous Tissue kit (BIO-RAD). Each isolation was performed in duplicate, and OD260/OD280 ratios were measured by Nanodrop (NanoDrop Technologies). Gene expressions of collagen type II and Aggrecan were measured by real time PCR (Lightcycler 480, Roche Diagnostics) to determine quality and quantity of mRNA transcripts. Data were normalized against three housekeeping genes (18S rRNA, UBC and YWHAZ).

For AC and M tissues, optimal results (high OD260/OD280 ratios and reproducible gene expressions) were found using the RNeasy Lipid kit, while the RNeasy Fibrous kit proved optimal for NP tissues. There were no significant differences between the two homogenization methods. We conclude that high-quality RNA can be isolated from cartilaginous tissues but different tissues require different kits.

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(313) Optimizing Arterio-Venous Pedicle Derived *In-Vivo* Angiogenesis of Prefabricated/Prelaminated Tissues via Angiosome Transfer Principles

Demirtas Y., Engin M.S., Arslan O., Karacalar A.

Ondokuz Mayıs University Medical School, Departments of Plastic, Reconstructive and Aesthetic Surgery, and Histology, Turkey

Introduction: One of the engineering applications is to place an avascular construct with an arterio-venous pedicle for neoangiogenesis. The purpose of this study is to optimize the technique for preparation of the pedicle in order to enhance vascularization.

Abstract Withdrawn

Materials and Methods: Fifteen rabbits were used for this study. Five of the animals were used in a pilot study to develop the protocol. During the study, thoracodorsal and lateral thoracic vascular pedicles on each side constituted 4 study groups. In group 1, the pedicle was desiccated. In group 2, the vessels were untouched. In group 3, the vessels were not desiccated, but dissected off the panniculus. In group 4, the pedicles were dissected and desiccated. In all of the groups, a 10×15 mm auricular cartilage was placed underneath the pedicles. After 2 weeks, the subjects were sacrificed via intra arterial lead oxide-gelatin injection and X-ray arteriograms were taken. VesSeg tool software was used to quantify the neovascularization of the cartilage grafts for area of vasculature, vessel intensity and vessel length. Also, specimens were harvested for histological analysis.

Results: Results indicate that groups 1 and 3 displayed better vascularization both qualitatively and quantitatively.

Discussion: Our results suggest that a degree of interference with the vessels significantly increases the induction of angiogenesis. However, it seems that excessively tampering with the vascular pedicles is equivalent to making no manipulation at all.

Conclusion: Either desiccation or dissection of a vascular pedicle before implantation of a construct may enhance neovascularization.

(314) Organic-Inorganic Hybrid Polyelectrolyte Scaffolds for Bone Tissue Engineering

Lim H.Y., Lee S.H., Chung H.Y., Ghim H.D., Shin H.I., Choi J.H.

Kyungpook National University, Dept Advanced Materials Sciences and Engineering, Daegu, Republic of Korea

We prepared organic-inorganic hybrid polyelectrolyte scaffolds constituted of chitosan/hydroxapatite (HAp)/alginate by two step lyophilizing method and evaluated them as scaffolds for artificial bone production.

This novel scaffold exhibited significantly improved mechanical and biological properties. According to SEM observation, the scaffolds have a high porosity with 120~150 µm of pore size. Well dispersed HAp particle increased tensile strength. Cell culture was assessed with KUSA-A1 osteoblast like cell. Hybridization of HAp and alginate improved biocompatibility, viability, metabolic activity. Also, the *in-vivo* study supported highly enhanced mineralization.

The result supports that chitosan/HAp/alginate hybrid polyelectrolyte scaffolds are suitable for a potential application in orthopedic bone regeneration.

(315) Orthogonal Scaffold of Magnetically Aligned Collagen Lamellae for Corneal Stroma Reconstruction

Torbet J.^{1,2}, Malbouyres M.¹, Builles N.³, Justin V.³, Roulet M.¹, Damour O.^{1,3}, Oldberg A.⁴, Ruggiero F.¹, Hulmes D.J.S.¹

¹Institut de Biologie et Chimie des Protéines, CNRS/Université Claude Bernard Lyon 1 UMR 5086, IFR 128 Biosciences Lyon-Gerland, Lyon, France

²Grenoble High Magnetic Field Laboratory, CNRS, Grenoble, France

³Banque de Cornées des Hospices Civils de Lyon, France

⁴Department of Cell Biology, University of Lund, Sweden

The creation of 3D scaffolds that mimic the structure of physiological tissue required for normal cell function is a major bio-engineering challenge. For corneal stroma reconstruction this necessitates the creation of a stroma-like scaffold consisting of a stack of orthogonally disposed sheets of aligned collagen fibrils. This study demonstrates that such a scaffold can be built up using magnetic alignment. By allowing neutralized acid soluble type I collagen to gel in a horizontal magnetic field (7 T) and by combining a series of gelation-rotation-gelation cycles, a scaffold of orthogonal lamellae composed of aligned collagen fibrils has been formed. Although initially dilute the gels can be concentrated without noticeable loss in orientation. The gels are translucent but their transparency can be greatly improved by the addition of proteoglycans to the gel-forming solution. Keratocytes align by contact guidance along the direction of collagen fibrils and respect the orthogonal design of the collagen template as they penetrate into the bulk of the 3-dimensional matrix. The scaffold is a significant step towards the creation of a corneal substitute with properties resembling those of native corneal stroma.

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(316) Osteoblast Activity is Affected by Collagen and GAG Concentrations and Crosslinking Temperature

Tierney C.M., Jaasma M.J., O'Brien F.J.

Royal College of Surgeons in Ireland, Department of Anatomy, 123 St Stephens Green, Dublin 2, Ireland

Collagen-glycosaminoglycan (GAG) scaffolds are currently used in many tissue engineering applications including skin, nerve and cartilage (1, 2). The aim of this study was to optimise the collagen-GAG scaffold for use in bone tissue engineering by modifying collagen and GAG concentrations and crosslinking density to determine their effects on cellular activity.

Scaffolds were made as previously described (3). Scaffolds were divided into two groups: [1] Collagen Scaffolds: 0.25%, 0.38%, 0.5%, 0.75% and 1% collagen (w/v) and [2] GAG Scaffolds: 0%, 0.05%, 0.1% and 0.2% GAG (w/v). Scaffolds were crosslinked to improve their mechanical properties by a dehydrothermal process at temperatures of either 105°C or 150°C. Scaffolds were then seeded with 2×10⁶ MC3T3-E1 osteoblasts and assessed for metabolic activity (alamarBlue™) and cell number (Hoechst 33258) at 24, 48 and 168 h post-seeding.

The scaffold variants crosslinked at 105°C showed increased metabolic activity and cell number with culture duration, and the 0.25%, 1% collagen and 0.2% GAG showed the greatest increase. The scaffold variants crosslinked at 150°C showed the greatest increase in both metabolic activity and cell number on the 0.75%, 1% collagen and 0.2% GAG constructs. Results indicate that increasing scaffold content and mechanical stiffness increases osteoblastic activity. These scaffolds will be subjected to long-term

experiments to examine the effects of composition and cross-linking on bone formation and mineralisation.

References

1. Yannas I.V., et al. PNAS USA.1983; 86:933–937.
2. Farrell E., et al. Tissue Engineering. 2006; 12:459–468.
3. O'Brien F.J., et al. Biomaterials. 2004; 25: 1077–1086.

(317) Osteoblast-like Cells' Response to Nanostructured Biomimetic Coatings

Caneva Soumetz F., Pastorino L., *Ruggiero C.

University of Genoa, Department of Communication, Computer and System Sciences—DIST, Via Opera Pia 13, Genova, 16145, Italy

The development of biomimetic constructs, based on the combination of suitable materials and biomolecules, is critical to elicit specific cellular responses with which to improve the success of any tissue repair strategy (1). Nanostructured biomimetic coatings containing fibronectin, an adhesive glycoprotein of the extracellular matrix, have assembled by means of the LBL technique on the surface of glass and of Nickel/Titanium (NiTi). In order to evaluate the potential of the developed coatings in bone tissue engineering, their influence on MG63 human osteoblast-like cells was investigated in terms of growth, dispersion and morphology.

Cell proliferation, which was evaluated by detaching and counting the number of adherent cells over time, resulted to be enhanced on the surface of the biomimetic coatings. Moreover, cell dispersion and morphology resulted to be very similar to those detected on the surface of standard tissue culture plates. The obtained results show that the developed coatings have a high potential for the engineering of the surface properties of biomedical implants and for the optimization of their integration with the surrounding tissues. The LBL technique is suitable for such purposes and in addition it is simple, environmental friendly and suitable for mass production of ultrathin biocompatible coatings (2).

References

1. Desai T. A. (2000). Micro- and nanoscale structures for tissue engineering constructs. Med. Eng. Phys. 22:595–606.
2. Lvov Y. et al. (1995). Assembly of multicomponent protein films by means of electrostatic layer-by-layer adsorption. J. Am. Chem. Soc. 117:6117–6123.

(318) Osteopontin Upregulation in a Compression Bioreactor Using Two Different Scaffolds

Sittichockechaiwut A., Brown L.E., *Reilly G.C.

Centre for Biomaterials and Tissue Engineering and Kroto Research Institute, University of Sheffield, S3 7HQ, United Kingdom

We have established a model to investigate the effects of mechanical loading on matrix production by bone cells in 3D. Cells are seeded in porous scaffolds and cultured in static conditions for at least 5 days. Cell-seeded scaffolds are subjected to bouts of mechanical loading at specified strains and frequencies in a sterile fluid filled chamber (Bose Electroforce 3200). In preliminary experiments 2 hours of compressive loading at 5% strain and 1 Hz

frequency caused a significant ($p < 0.05$, two sample t-test, $n = 4$) increase in collagen production as measured by sirius red. Loaded samples contained 50% more collagen than non-loaded controls, the relative difference was not changed by increasing the number of loading bouts. However, reducing the loading period to 1 or 0.5 hrs produced no detectable difference in collagen production by the cells. To assess loading induced changes in matrix gene expression 2 types of culture were set up. 1) MLO-A5 cells in polyurethane foams (BritishVita). 2) MC3T3-E1 cells in collagen sponges (BD biosciences). In both experiments cells were cultured until day 5 then subjected to a single bout of 2 hours of loading at 5% strain, 1 Hz. In both experiments an increase in osteopontin mRNA was detectable in loaded samples by PCR. We have demonstrated that gene expression changes can be seen in different cell/scaffold combinations using our loading system. This model system will be useful for the optimisation of the mechanical environment in bioreactor based tissue engineering.

(319) Oxygen Generating Scaffolds for Tissue Regeneration

Harrison B.S., Eberli D., Lee S.J., Oh S.H., Ward C., Atala A., Yoo J.J.

Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, United States of America

Introduction: One of the significant challenges facing tissue engineering is the inadequate diffusion of oxygen to transplanted cells. In this study we investigated whether the diffusion problem could be solved by incorporating oxygen producing materials to generate endogenous oxygen. We fabricated a scaffold system that would provide sustained release of oxygen to cells and tissues for prolonged survival *in vivo*.

Methods: Oxygen producing films were prepared by combining PLGA with sodium carbonate peroxyhydrate (SPO). To assess oxygen release, films were placed in water and the rate of oxygen release was measured. To demonstrate the efficacy *in vivo*, the films (20×10 mm) were implanted in an ischemic skin flap model (mouse), and graft necrosis was measured by skin discoloration and digital image analysis.

Results: Successful incorporation of SPO within the polymers was confirmed by the release of oxygen gas bubbles when placed in water. The analysis of graft necrosis shows a significant benefit for the SPO group in the early time points up to 3 days.

Conclusions: We demonstrate that oxygen diffusion limitation can be solved by incorporating oxygen producing compounds into scaffolds. The polymers containing SPO are able to prevent necrosis and prolong tissue viability *in vivo*. This novel system may lead to an accelerated clinical translation of tissue engineering technology.

(320) Patterning of Endothelial Cells for Designed Capillary Formation

Henmi C.¹, Nakamura M.^{1,2}, Nishiyama Y.¹, Watanabe A.², Iwasaki Y.², Morita I.³, Yamaguchi K.¹, Mochizuki S.¹, Takiura K.¹, Nakagawa H.¹

¹Kanagawa Academy of Science and Technology, Think E building, 1-23 Minamiwatarida, Kawasaki-ku, Kawasaki-city, Kanagawa, 210-0855, Japan

²Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Japan

³Department of Cellular Physiological Chemistry, Tokyo Medical and Dental University, Japan

Vascularization of engineered tissue is one of the most important issues in the development of tissue engineering. Whole of the engineered tissues must be perfused to periphery with oxygen and nutrients to maintain their viability, however, the method to control vascularization is very limited, especially *in vitro* tissue engineering.

Then, we tried to make designed capillary vessels using patterned vascular endothelial cells (ECs), because we supposed that the techniques to make designed capillary vessels will be effective to fabricate and develop vascularization in the various engineered tissues.

First of all, we tried to make patterned ECs using our original inkjet printer [1]. 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer, which is known as one of the biocompatible polymers to inhibit attachment of the cell and proteins, was used for inkjet printing. We printed linear patterns with MPC polymer at 250 μm interval onto cell culture disks. Then, the ECs were seeded and incubated on the polymer patterned disk.

The cellular attachment at linear pattern was observed. ECs were adhered only onto the non-polymer-coating region. A line width of cellular attachment area was also approximately 250 μm . Time lapse microscopic observation showed that ECs were not adhered within the polymer coating region at all.

By using polymer inkjet technology, we achieved polymer patterned coating and clear patterned ECs could be obtained. Next we are trying to develop patterned capillary vessels.

Reference

1. Nakamura M., Nishiyama Y., Henmi C., et al. Inkjet bioprinting as an effective tool for tissue fabrication. Proceeding of DF 2006, 89–92, 2006.

(321) Periodontal Regeneration by Tissue Engineered Autologous Bone Marrow Mesenchymal Stem Cells

Chang S.C.N., Lin T.M., Chung H.Y., Jeng L.B.

Department of Plastic Surgery, China Medical University Hospital, Taichung, Taiwan

Due to the more complexity of periodontal alveolar bony regeneration, various procedures have been developed and used. Human recombinant bone morphogenetic protein 2 (rhBMP-2) *in vivo* showed a significant potential for stimulating periodontal bone regeneration but with some various unfavorable results including limited regeneration of cementum and periodontal ligaments combined with local root resorption or ankylosis.

To evaluate the regeneration of periodontal apparatus in critical mandibular alveolar bony defects by BMP-2 gene engineered autologous bone marrow mesenchymal stem cells (MSCs).

Twelve critical mandibular periodontal defects were surgically created over the premolar area combined with root denuded at

least 4mm below cemento-enamel junction (CEJ) in 6 matured male dogs. Bilateral mandibular defects were randomly assigned into 2 groups: BMP-2 gene engineered MSCs/polymer (BMP-2 group), and MSCs/polymer as control. Histological examination was performed at the end of study. Three-dimensional computerized tomography was utilized to calculate the total volume of bone regenerated.

Our data demonstrated that the regeneration of periodontal apparatus was significantly better in BMP-2 group. New cementum with Sharpey's fiber was observed on the instrumented denuded root surfaces in BMP-2 group. Incomplete healing with localized root surface resorption was noted in control groups. Larger amounts of bone were regenerated in BMP-2 group 8 weeks after implantation.

These results indicated that *ex vivo* BMP-2 gene engineered autologous MSCs enhanced both bone and periodontal apparatus regeneration in the mandibular critical periodontal defect in dog. Such a new approach was a suitable clinical application for better healing in bone and the periodontal apparatus.

(322) PHBV, PCL and PLGA Microsphere Support the Attachment and Proliferation of Goat and Human Mesenchymal Stem Cells

Ramlogan A.S., De Bruijn J.D.

Queen Mary University of London, School of Engineering and Materials Science, Engineering Building, Mile End Road, London, E1 4NS, United Kingdom

Mesenchymal Stem Cells (MSCs) hold much promise in regenerative medicine, tissue engineering and cell therapy strategies. By virtue of their multipotency, MSCs can be stimulated to form mesenchymal tissues such as bone, cartilage, fat and muscle. Hence, MSCs may be used to repair or replace damaged tissues. A major challenge to the clinical success of MSC-based strategies is the ability to get sufficient numbers of MSCs within an acceptable time frame.

Herein, we evaluated whether polymeric microspheres composed of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [PHBV], poly(caprolactone) [PCL], polyglycolide-co-lactide [PLGA] and Cytodex 1 can support the attachment and expansion of human MSCs.

Bone marrow derived MSCs were harvested from the acetabulum of a 42 year old male and grown as described previously [1]. Passage 1 MSCs were seeded onto the various microspheres (2000 cells/cm²) and the doubling times were evaluated based on Alamar blue measurements over a 5 day period. Cytodex 1 was used as the control. The shortest doubling time (71.8 ± 12.5 hours) was found to be on PHBV microspheres. PLGA (145.0 ± 11.4 hours) and PCL (556.8 ± 14.0 hours) had significantly longer doubling times. Interestingly, a parallel experiment showed the opposite with goat MSC expansion on the same microspheres; the shortest doubling time was on PLGA (42.4 ± 12.6 hours) and the longest on PHBV (75.2 ± 11.9 hours).

Reference

1. Mendes, S.C. et al (1998). *In vivo* bone formation by human bone marrow cells: effect of osteogenic culture supplements and cell densities. Journal of materials science 9, 855–858.

(323) Platelet-Lysate as Substitute of FCS Improves the *In Vivo* Bone Forming Capacity of Mesenchymal Stromal Cells on Scaffolds

Prins H.J.¹, Rozemuller H.¹, Slaper-Cortenbach I.², Vonk-Griffioen S.², Martens A.¹

¹Department of Immunology, University Medical Center Utrecht, The Netherlands

²The Utrecht Center for Gene and Cell Therapy, University Medical Center Utrecht, The Netherlands

A comparison was made between culturing mesenchymal stromal cells (MSCs) in the presence of human platelet lysate (PL) or with fetal calf serum (FCS) for their expansion, differentiation and *in vivo* bone forming capacity. Bone marrow from six healthy donors was ficoll separated and mononuclear cells were cultured in α -MEM/5% PL or in DMEM-LG/10% FCS and characterized for CD marker expression (FACS) and differentiation potential.

Third passage cells from both cultures were seeded onto biphasic calcium phosphate particles ($n = 6$). Osteogenic differentiation was induced by incubating the cells with medium containing dexamethasone for 7 days. Thereafter, hybrid constructs were implanted subcutaneously on the back of 6 nude mice. After 6 weeks constructs were explanted and thin sections were prepared and stained for bone histomorphometry.

MSCs cultured with PL resulted in a fourfold higher expansion than MSCs cultured with FCS. The MSC phenotype was identical and the MSCs differentiated into all 3 lineages (osteoblasts, adipocytes and chondrocytes). No bone formation was observed in control scaffolds without cells; FCS cultured cells resulted in minimal bone deposition in 5/6 mice; PL cultured cells showed substantial bone formation in 6/6 mice.

For clinical application MSCs can be expanded under GMP conditions using PL to circumvent the usage of animal serum. These culture conditions yield much higher numbers of MSCs with a similar phenotype and *in vitro* multipotency. The MSCs expanded with PL resulted in increased bone formation *in vivo*. The culture procedure that we developed can play an important role in bone tissue engineering for human application.

(324) Poly Lactic Acid Scaffold Augmented with Human Bone Marrow Stromal Cells as a Bone Graft Extender in Impaction Bone Grafting

Bolland B.J.¹, Kanczler J.M.¹, Ginty P.J.², Shakesheff K.M.², Dunlop D.G.¹, Oreffo R.O.C.¹

¹Bone & Joint Research Group, University of Southampton, Southampton, SO16 6YD, United Kingdom

²School of Pharmacy, University of Nottingham, United Kingdom

Concerns over disease transmission, high costs and limited supply of allograft have led to interest in synthetic grafts in the field of Impaction Bone Grafting (IBG). Poly (lactic acid) (PLA) grafts are attractive due to their biocompatibility, established safety and versatility. This study examined the potential of PLA scaffolds augmented with human bone marrow stromal cells (HBMSC) in IBG.

Impacted morsellised PLA grafts seeded with HBMSC were incubated *in vitro* under osteogenic conditions and implanted into the subcutis of nude mice *in vivo*, for 4 weeks and compared to PLA alone.

Cell tracker green and ethidium homodimer-1 staining confirmed HBMSC survival post impaction *in vitro* and *in vivo*. *In vitro* there was a significant increase in DNA content ($p < 0.001$), specific alkaline phosphatase activity ($p < 0.001$) and a significant increase in shear strength and interparticulate cohesion in PLA/HBMSC samples compared to impacted PLA alone. Type I collagen immunohistochemistry confirmed the osteogenic cell phenotype. *In vivo* studies showed a significant increase in penetrating blood vessel number and volume together with a significant relative increase in new bone formation in the PLA/HBMSC constructs compared to PLA alone ($p = 0.02$, $p = 0.004$) using microcomputer tomography.

HBMSC seeded onto PLA withstand femoral impaction continue to proliferate and differentiate along the osteogenic lineage, and PLA/HBMSC constructs *in vitro* offer a mechanical advantage over PLA alone. PLA/HBMSC constructs *in vivo* induce neovascularisation and new bone formation. These studies demonstrate that PLA is a biological and mechanical advantageous bone graft extender for use in IBG, with extensive versatility for further modification and augmentation.

(325) Polycaprolactone-based Biocomposite Scaffolds for Tissue Engineering: Assessment of Macroporosity Using Micro Computed Tomography (micro-CT)

Wang Y.¹, Chang H-I.², Wertheim D.F.³, Jones A.S.⁴, Coombes A.G.A.¹

¹School of Pharmacy and Chemistry, Kingston University, London, Kingston upon Thames, Surrey KT1 2EE, United Kingdom

²School of Pharmacy, The University of Queensland, Brisbane, QLD 4072, Australia

³Faculty of Computing, Information Systems and Mathematics, Kingston University, London, Kingston upon Thames, Surrey KT1 2EE, United Kingdom

⁴Australian Key Centre for Microscopy and Microanalysis, University of Sydney, NSW 2006, Australia

⁵Sutton Arthritis Research Laboratories, University of Sydney, NSW 2065, Australia

Pore interconnections within tissue engineering scaffolds are essential to allow, at one level, nutrient supply, metabolite dispersal and cell signaling. However, successful cell colonization requires an additional level of control over macropore size, geometry and connectivity. Micro-CT has been shown to provide a powerful tool for assessing the extent to which scaffolds meet the latter design criteria and for evaluating subsequent tissue/scaffold interaction. Polycaprolactone-based biocomposites containing high loadings (30–40% w/w) of water-soluble particulates have been produced by precipitation casting for application as 3-D scaffolds. Extraction of the water-soluble phase results in a macroporous surface and internal architecture which reflects the shape and size range of incorporated particles (e.g. 90–125 μ m). Release studies confirmed that the pore networks are sufficiently developed to permit efficient diffusion of small molecules (lactose Mw 360.3) and macromolecular species (gelatin Mw 20,000–30,000). Gradual release of almost 90% of a high protein load (30–40% w/w) was obtained over three weeks in PBS at 37°C, demonstrating a potential for efficient delivery of growth factors to improve tissue regeneration. Micro-CT provided a quantitative measure of the frequency distribution of equivalent pore diameters throughout the matrix, which

is useful for optimising scaffold design, production and performance. Micro-CT confirmed the presence of an extensive internal system of macropores but the virtual absence of a network of high volume, inter-pore channels required for cell infiltration of the material. Thus tissue integration would be confined initially to the biocomposite surface, for the designs investigated, with the core potentially providing a depot for controlled delivery of growth factors.

(326) Polyester and Collagen Scaffolds for Cornea Tissue Engineering

*Hasirci V., Vrana N.E., Zorlutuna P.

METU, BIOMAT, Department of Biological Sciences, Biotechnology Research Unit, Ankara, Turkey

*Present Address: Dublin City University, MPRC, Dublin, Ireland

Three different forms of polyester and collagen-based scaffolds, patterned films, foams, and film-foams were developed for use in cornea tissue engineering. Thickness, transparency, density, porosity, degradation and topography were studied using 3 different cell types, D407 retinal pigment epithelial cells, 3T3 fibroblasts, and human corneal keratocytes. Films were seeded separately with all the cell types and the foams with 3T3 fibroblasts and keratocytes. 3D film-foam constructs were seeded simultaneously with D407 (film side) and 3T3 cells (foam side) to mimic epithelial and stroma layers of cornea. SEM and immunofluorescence studies were carried out to evaluate cell distribution and orientation, to assess cell-to-cell contacts, ECM secretion, and cytoskeleton alignment. Foams produced had high porosities. The films were produced with a number of different patterns. Different rates of degradation were observed with different forms. Degradation and cell presence affected the transparency of the films. Cells proliferated on all scaffolds and aligned along the patterns on the films and secreted ECM. Fibronectin coating improved cell attachment and alignment. Mechanical tests showed that films have enough strength for use as corneal substitutes. Strength of keratocyte seeded films increased as cell number increased. In conclusion, several scaffolds were produced from biodegradable polymers and were shown to be suitable for corneal tissue engineering.

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(327) Polymer Synthesis and Processing for a Bioreactive Composite Scaffold in the VASCUPLUG Project

Volkman A., Schinkel G., Kelch S., Weigel T.

GKSS Forschungszentrum, Institute of Polymer Research, Kantstr. 55, 14513 Teltow, Germany

The VASCUPLUG project is concerned with designing a bio-reactive composite scaffold for improved vascular connexion of tissue-engineered products. The novel scaffold consists of three main components: foam, tubular structures and stimuli-sensitive gel with encapsulated bioactive substances.

The signal for the stimuli-sensitive gel to contract is intended to be a pH drop in the surrounding tissue, related to malnutrition of cells. The pH sensitive hydrogels were prepared by free radical

polymerisation and their pH dependent swelling behaviour was modified by complexation with polymeric additives. Since polysaccharides are already used in the context of biomedical applications and are known to be compatible with biological systems and are biodegradable, the synthesis of the hydrogels based on sodium alginate was established. The resulting gels are evaluated in relation to their cytotoxicity and biocompatibility.

Several polymers were examined for the production of the components like commercial poly(ϵ -caprolactone) (PCL), poly(L-lactide-co-glycolide) (PLGA), poly(p-dioxanone) (PDX) as well as polymers based on poly(acrylic acid) (PAA). Pressure quenching or thermally induced phase separation (TIPS) was applied in order to fabricate foam-like structures with interconnected pores. The tubular structures were prepared by non-solvent induced phase inversion (NIPS).

For the construction of the composite scaffold a tubular structure was formed with the pH-sensitive hydrogel by dip coating a hydrogel fibre. Then a highly porous structure was foamed around the filled fibre by TIPS or pressure quenching.

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(328) Polyurethane Scaffolds for Long Term Mechanical Loading in Musculoskeletal Tissue Engineering

Clarke D.A., Coll C., Drury P., Crawford A., Reilly G.C.

Centre for Biomaterials and Tissue Engineering, University of Sheffield, S3 7HQ, United Kingdom

Musculoskeletal tissue engineering involves using biodegradable polymer scaffolds as cell carriers. Mechanical stimulation of cells in scaffolds has been shown to increase matrix production; however, due to their poor mechanical properties biodegradable scaffolds are inappropriate for long-term mechanical loading experiments.

Polyurethanes (PU) are regarded as a suitable biomaterial for musculoskeletal applications due to their biocompatibility and excellent durability. We analysed the efficacy of differing polyurethane compositions for mechanical loading of musculoskeletal cells in 3D. We analysed a commercially available PU foam, 3000 Mw propylene oxide soft segment and toluene diisocyanate (TDI) hard segment, and four "home-made" poly(urethane-co-urea) foams, composed from a TDI hard segment and mixed soft segments formed from two polyols: VOR, a 3000 Mw glycerol based polyol and REFC, a 2690 Mw mixed polyether polyester polyol in ratios of 100:0, 75:25, 50:50 REFC:VOR and 100:0 VOR:REFC.

MTS viability assays of foam seeded with the MC-3T3 cell line indicated that each scaffold supports cell growth, with VOR:REFC 50:50 foam supporting the highest cell number. Tensile mechanical testing of samples in tension (Bose-Enduratec 3200) showed 100% REFC to have the highest modulus at 29 kPa, with 75% REFC lowest at 7 kPa.

1×1 cm samples of VOR 50:50 foam were seeded with MC-3T3s at 500,000 cells per scaffold, compressively loaded for 2 hrs at 1Hz, 5% strain on days 5 and 7 of culture. MTS assays on day 12 of culture indicated an increase in cell number in loaded compared to non-loaded samples.

Ongoing work includes the fabrication of electrospun polyurethane scaffolds to allow comparison of foam and fibre environments.

(329) Porous 3D-Scaffold for Cell Culturing and Transplantation Based on 3-hydroxybutyrate-co-valerate

Nemets E.A., Markovtseva M.G., Egorova V.A., Sevastianov V.I.

Research Institute of Transplantology and Artificial Organs, Moscow, Russian Federation

Introduction: One of the key problems of cell culturing and transplantation is the development of suitable porous 3D scaffolds. The use of biodegradable polymers for 3D carrier preparation holds great promise due to their high biocompatibility and controllable biodegradation rate. Recently the clinical trials have been finished for a novel implantable 2D-matrix ElastoPOB[®] from poly (hydroxybutyrate-hydroxyvalerate) copolymer with increased surface hydrophilicity. The aim of this research was the development of 3D-scaffolds using composite materials of ElastoPOB[®].

Materials and Methods: Three-dimensional porous scaffolds ElastoPOB[®]-3D were prepared by means of well-established salt leaching technique. The degradation rate of samples was evaluated in model solution (PBS, pH = 7.4, 370°C) during a 3-month period. L929 mouse fibroblasts were cultured on ElastoPOB-3D[®] for 3–10 days. The results were analyzed using SEM and luminescent microscopy.

Results and Discussion: ElastoPOB[®]-3D represented a highly porous scaffold (porosity of $95 \pm 2\%$) with homogeneous pore structure consisting of macro- and micropores. Macropores with size from 100 to 250 μm have been interconnected by micropores ranged from 5 to 20 μm . After 3 months of biodegradation the scaffolds lost about 30% of their initial weight. On the 3rd day of fibroblast culturing separate adhering spindle-shaped cells were observed on scaffold surface, confirming their high viability. By the 7th day the number of cells on ElastoPOB[®]-3D surface increased significantly, and on the 10th day a cell monolayer is formed. The obtained experimental data allow hope for the perspective use of ElastoPOB[®]-3D as biodegradable implantable scaffold for creation of internal hybrid organs.

(330) Porous Film Grafts Allow Support, Transfer and Delivery of Live Human Cells to Wounded Model Mice

McMillan J.R., Akiyama M., Tanaka M., Yamamoto S., Abe R., Kodama K., Shimomura M., Shimizu H.

Department of Dermatology, Graduate School of Medicine and Creative Research Initiative Sousei, Faculty of Science, Hokkaido University, Sapporo, Japan

We have previously demonstrated that porous poly-(epsilon-caprolactone) films with regularly spaced, controlled pore sizes provide adhesion and support for cultured dermal fibroblasts. We have determined the effects of applying various sized porous films ($n = 3$ for each treatment) on 4 mm punch biopsy wounded mice to assess wounding response. Films with pores ranging in size from 3–20 microns, elicited a mild lymphocytic and foreign body perifollicular immune response, regardless of pore size but this treatment failed to significantly shorten wound healing time or

increase the rate of wound closure. By 21 days after wounding the grafted porous films had become fully incorporated into or completely biodegraded in the wounded tissue. Finally, we assessed the proof of principle that live cultured fibroblasts can be delivered using porous films and sustained in model SCID mouse wounds. Human fibroblasts (30,000 cells) were subconfluently cultured on 5 micron porous films. These cell/film combinations were then transplanted onto wounded mice but failed to significantly affect wound healing. However, these transplanted fibroblast cells were readily detected using anti-human HLA antibodies in wounded SCID mice skin 21 days after treatment, when the wounds had completely healed. Taken together, these data demonstrate for the first time the feasibility of using porous films to deliver living human cells into skin wounds as part of our aim to use cell therapy to improve the wound healing response.

(331) Porous Micropatterned Scaffolds to Control Tissue Organization

Papenburg B.J.¹, Rouwkema J.², Stamatialis D.¹, De Boer J.², Van Blitterswijk C.A.², Wessling M.¹

¹Membrane Technology Group, ²Department of Tissue Regeneration Institute for BioMedical Technology (BMTi), Faculty of Science and Technology, University of Twente, Enschede, The Netherlands

When designing scaffolds, a great deal of attention should be given to: (I) nutrient supply, provided by inner-porosity, (II) micropatterning, to promote cell orientation improving tissue functionality.

In contrast to previous studies addressing only porosity or micropatterning, we developed 2-D porous polymer sheets with enclosed micropattern [1]. In fact, we showed preparation and characterization of highly porous micropatterned sheets fabricated by the one-step fabrication method called Phase Separation Micromolding (PS μ M). PS μ M is based on immersion precipitation on a micropatterned mold replicating the micropattern into the polymer sheet during solidification. Optimized poly(l-lactic acid) sheets (porosity > 80%) had high glucose diffusion levels indicating suitable porosity considering nutrient transport. Mouse myoblasts, C2C12, showed alignment of 60–81% induced by various micropattern-architectures confirming control of tissue organization through micropattern design.

In this work, we focus our attention on (I) multi-layer stacking of these micropatterned porous sheets and (II) co-culture of C2C12 and HUVEC (human umbilical vein endothelial cells) aiming to obtain vascularized organized tissue in a 3-D scaffold. Nutrient transport through the stacks is studied, both experimentally as well as modelled. The influence of various polymers as well as micropattern design on tissue growth and organization is evaluated. Furthermore, C2C12 and HUVEC's are co-cultured on the stacked micropatterned sheets to investigate vascularization within the stacks.

Reference

1. Papenburg, B.J., Vogelaar, L., Bolhuis-Versteeg, L.A.M., Lammertink, R.G.H., Stamatialis, D., Wessling, M., (2007) One-step fabrication of porous micropatterned scaffolds to control cell behavior, *Biomaterials* 28, 1998–2009.

(332) Porous Tubular Scaffolds Based on Flexible and Elastic TMC (Co)polymers

Song Y.¹, Weekamp H.H.^{1,2}, Kamphuis M.^{1,2}, Zhang Z.¹, Vermes I.^{1,2}, Poot A.A.¹, Grijpma D.W.¹, Feijen J.¹

¹Institute for Biomedical Technology (BMTI) and Department of Polymer Chemistry and Biomaterials, Faculty of Science and Technology, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands

²Department of Clinical Chemistry, Medical Spectrum Twente Hospital, Enschede, P.O. Box 50000, 7500 KA, The Netherlands

Introduction: For tissue engineering of small-diameter blood vessels, biodegradable, flexible and elastic porous tubular structures are most suited. The applicability of poly(trimethylene carbonate) (PTMC), random copolymers of TMC and ϵ -caprolactone poly (TMC-CL), and networks based on these polymers as scaffolding materials was investigated.

Methods: TMC-based (co)polymers were synthesized by ring-opening polymerization. Tubular structures were prepared by dipping glass mandrels in polymer solutions containing dispersed, sieved sugar particles, followed by γ -irradiation and cross-linking, and leaching. For mechanical- and biocompatibility tests, films of different thicknesses were prepared by compression molding, solvent casting, and spin-coating.

Results and Discussion: PTMC and poly(TMC-CL) are flexible materials, with E-modulus values below 10 MPa and elongations at break higher than 500%. After γ -irradiation in vacuo at 25–100 kGy, networks with gel contents up to 73 wt% were obtained. The networks showed excellent creep resistance under static and dynamic loading conditions.

Good cell attachment and proliferation behavior of mesenchymal stem cells, endothelial cells, and smooth muscle cells on polymer films and networks was observed. In lipase solutions, the films degraded substantially within one month by surface erosion.

Porous tubular structures, with pore sizes in the range of 80–130 μ m and a porosity of approximately 85%, could readily be prepared. A pulsatile bioreactor that allows mechanical stimulation of smooth muscle cells and endothelial cells seeded in the porous structures is being constructed.

Conclusions: TMC-based (co)polymers and networks are flexible, elastic, biocompatible, and biodegradable. Porous tubular scaffolds based on these materials have much potential in tissue engineering of small diameter blood vessels.

(333) Potential of Synovial Fluid Stem Cells for Cartilage Engineering

Crawford A.¹, Jones E.A.², English A.², Mundy J.¹, Henshaw K.², Corscadden D.², Emery P.², McGonagle D.², Hatton P.V.¹

¹Centre for Biomaterials and Tissue Engineering, School of Clinical Dentistry, University of Sheffield, United Kingdom

²Rheumatology Research, University of Leeds, CSB Room 5.24, St James Hospital, Leeds, United Kingdom

Background: Bone marrow (BM) mesenchymal stem/progenitor cells (MSCs) are potentially useful for engineering cartilage tissue. However, chondrogenic cells derived from BM-MSCs can undergo hypertrophy and mineralization *in vivo* (1). The specialized microenvironments or niches containing stem cells govern their function. Therefore, MSCs within a “joint niche” may have higher chondrogenic and lower osteogenic potential than BM-MSCs.

Aim: To compare isolated SF-MSCs from normal SF and matched BM-MSCs for their utility with for engineering hyaline cartilage.

Methods: Matched bovine BM and SF-MSCs clones were established (2). Polyglycolic acid scaffolds were seeded with clonal or polyclonal BM- or SF-MSCs (3) and cultured for 42 days in classical chondrogenesis- or osteogenesis-promoting media to stimulate appropriate differentiation (2, 4). Construct extracellular matrix was examined by histochemical, immunochemical and biochemical methods.

Results: Under chondrogenic conditions, SF-MSC constructs were larger (12.81 ± 2.44 mg wet weight, $n = 4$ clones) than BM-MSC constructs where only 1 of 3 reached a similar size (wet weights 13.79 mg, 3.16 mg and 3.19 mg, $n = 3$ clones). All the constructs produced extracellular matrix containing collagen II and proteoglycan. Calcium deposition was absent. Interestingly, in osteogenic culture conditions, no mineralization was observed in the SF-MSC constructs whereas calcium deposition was observed in BM-MSC constructs.

Conclusion: SF-MSCs represent an attractive cell source. Their apparently higher chondrogenic and lower osteogenic potential than BM-MSCs suggests that SF-MSCs may have utility for cartilage repair therapies in trauma and arthritis.

References

1. Pelttari K, et al. *Arth & Rheum* 2006;54:324.
2. Jones EA, et al. *Arth & Rheum* 2004;50:817.
3. Crawford A and Dickinson SC. *Meth Mol Biol* 2004;238:147.
4. Crawford A, et al. *Rheumatology*. 2006;45:1529.

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(334) PPF/DEF-HA Composite Scaffold Using Micro-stereolithography

Lee J.W., Lan P.X., Seol Y.J., Cho D.W.

Department of Mechanical Engineering, POSTECH, Pohang, Gyeongbuk 790-784, South-Korea

Introduction: Many researchers have reported the merits of micro/nano-scale composite structures for the promotion of cell proliferation [1, 2]. However, conventional methods of scaffold fabrication cannot control the shape of composite structures. However, solid free-form fabrication (SFF) can control the shape of composite structures, and among SFF technologies, micro-stereolithography (MSTL) has the highest resolution. We used MSTL to fabricate a micro/nano-scale composite scaffold containing hydroxyapatite

(HA) nano-powder, and the suitability of this material for cell adhesion was evaluated.

Methods: Poly(propylene fumarate)(PPF)/diethyl fumarate(DEF) composite possesses compressive mechanical properties that are similar to those of human trabecular bone [3]. And HA is one of the main components of bone. Briefly, PPF was synthesized via a condensation reaction, according to Gerhart et al. [4]. After synthesis, DEF was added to reduce the viscosity. Finally, a photoinitiator (dimethoxy-phenyl-acetophenone) and HA were mixed with the synthesized photopolymer. MSTL was performed using an Ar ion laser, an x-y-z stage, and optical components. This method was used to fabricate 3-D scaffolds by solidifying layers.

Results and Discussion: Scaffolds containing micro/nano structures were successfully fabricated using MSTL and PPF/DEF-HA. The pores and lines of the fabricated scaffold were regular and all the pores were connected. HA powder generated the micro/nano-size topology well. After scaffold fabrication, MC3T3-E1 cells were seeded within the scaffolds and cell adhesion was observed. Our results indicate that scaffolds containing HA powder can be applied to bone tissue regeneration.

References

1. Y. Wan, et al.,(2005), *Biomaterials*(26), 4453–4459.
2. G. Wei, et al.,(2004), *Biomaterials*(25), 4749–4757.
3. J.P. Fisher, et al.,(2002), *Biomaterials*(23), 4333–4343.
4. D.D. Frazier, et al.,(1997), *J Biomed Mater Res*(35), 383–389.

(335) Prelamination of Keratinocytes, Fibroblasts, Adipocytes and EPCs to Create an Entirely Autologous, Full Thickness and Vascularised Skin Substitute: Are We There Yet?

Van den Berge S., Hendrickx B., Dickens S., Vranckx J.J.

Laboratory of Plastic Surgery and Tissue Engineering Research, Department of Plastic & Reconstructive Surgery, KULeuven University Hospital, Katholieke Universiteit Leuven, Herestraat 49, B-3000, Belgium

Skin substitutes come in a dizzying variety nowadays. None, however, has managed to perfectly replace full thickness skin. Rejection and infection are, on more than one occasion, the culprits for skin substitute failure. It is commonly believed that the exclusion of foreign-origin materials and an improved vasculature in the skin substitute are factors that may aid substantially in achieving better results. We therefore focus on constructs that come with the potential for vascularisation, and are made up entirely of the host's own expanded cells.

Fibroblasts, keratinocytes and adipocytes were isolated from small skin fragments and expanded *in vitro*. After 3–4 passages, the cells were left to proliferate and form multi-layers for 3–4 weeks in a differentiation medium consisting of a DMEM/Ham's F12 mixture, supplemented with foetal calf serum or a serum replacement and various nutrients and growth factors. In the meantime, endothelial progenitor cells (EPCs) were harvested from peripheral blood samples, isolated and cultured into stable cell lines of late outgrowth EPCs (with a large proliferation capacity).

The next step was a classic "mix 'n' match": laminated fibroblast and keratinocyte sheets were superimposed, with different concentrations of EPCs sandwiched between them, and adipose cell

(multi)layers underneath, to investigate the cells' behaviour, proliferation and differentiation, and the EPCs' ability to form vessel-like tubular structures.

The aim of this research project is to further investigate the concept of prefabrication and -lamination for tissue engineering of full-thickness skin substitutes.

(336) Preliminary Study of *In Vitro* Niche Effect on Differentiation of Rat Bone Marrow Stem Cells to Cardiomyocytes-Like Cells

Miskon A.^{1,3}, Terada D.², Ehashi T.², Fujisato T.², Mahara A.¹, Uyama H.³, Yamaoka T.¹

¹Department of Biomedical Engineering, Advanced Medical Engineering Center, National Cardiovascular Center Research Institute, Japan

²Department of Tissue Engineering, National Cardiovascular Center Research Institute, Japan

³Department of Chemical Engineering, Osaka University, Japan

The adult heart does not regenerate after injury because the cardiac myocytes are terminally differentiated and lost their growth activity. Injection of the bone marrow stem cells into infarcted region is one of the most promising solutions proposed, and recent evidence has suggested that stem cell can differentiate into cardiomyocyte either *in vivo* or *in vitro*. However the efficiency of the differentiation process is still very low. In this study, we investigated the effect of various culture conditions including differentiation medium, cell culture matrices, and culture system (suspension or monolayer culture and static culture or dynamic culture) on the differentiation of rat mesenchymal stem cells (rMSCs) to cardiomyocytes-like cells under *in vitro* condition. First, the effect of 5-azacytidine, vitamin-C and human basic fibroblast growth factor (b-FGF) added to Dulbecco's modified Eagle's medium-low glucose (DMEM-LG) in static culture was determined. The rMSCs were obtained from femurs and tibias of male Sprague-Dawley (SD) rats (2-week-old) using our own method. rMSCs (6.0×10^5) at the third passage as seeded in the culture dish were then exposed to differentiation medium for 24 hours on day-3 of culture. The RT-PCR result shows that the expression of α -actin gene has increased to a peak value after 2 weeks induced by 5-azacytidine, vitamin-C, and b-FGF but the expression of MEF2C gene was low. Then, we treated the rMSCs suspended in DMEM containing 5-azacytidine, vitamin-C, and b-FGF. As a result, the expression of α -actin gene was forty times higher than control, whilst the MEF2C was forty times higher than the control. The effect of the other "niches" will be also discussed.

(337) Preparation and Characterization of Cornea Decellularized by Ultra High Pressurization

Kimura T., Funamoto S., Hashimoto H., Sasaki S., Mochizuki M., Fujisato T., Kobayashi H., Kishida A.

Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo, Japan

Department of Ophthalmology, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo, Japan

Department of Biomedical Engineering, Osaka Institute of Technology, Asahi-u, Osaka, Japan
Biomaterials Center, National Institute for Materials Science, Tsukuba, Ibaraki, Japan

Although corneal transplantation is an effective treatment for serious corneal diseases, the lack of supply is one of important problems. Also, inflammatory rejection has often occurred. In the present study, we developed the decellularized cornea by ultra high pressurization as novel artificial cornea. Porcine corneas were pressurized at 4,000 to 10,000 atm and 10 to 30 degrees C for 10 min, and then immersed in culture medium containing DNase I, antibiotics for 72 hours to remove the cellular components (UHP method). The decellularized corneas were subjected to histological study (H-E staining). The transparency, thickness and mechanical strength of them were investigated. When a porcine cornea was hydrostatically pressed at 4,000 or 10,000 atm at 10°C for 10 min, semi-transparent cornea was obtained by pressurization at 4,000 atm and 10°C. The transparency of cornea was decreased under the higher pressure and temperature condition. The swelling of them was observed for the washing process. By H-E staining, the complete removal of epithelial and stromal cells was confirmed in all of the pressurized corneas. The superstructure of their collagen fibrils was relatively maintained. Furthermore, the transmittance of the decellularized corneas by UHP method was recovered by the immersion of them in glycerol and the mechanical property similar to native cornea was shown. These results indicate that the decellularized cornea by UHP method would be useful as corneal scaffold for regeneration.

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(338) Preparation and Evaluation of an Injectable Hyaluronan Hydrogel for Therapeutic Applications

Hilborn J., Bergman K., Ossipov D., Bowden T., Engstrand T.

Materials Chemistry, Uppsala University, 751 21 Uppsala, Sweden

A method for the preparation of hyaluronan derivatives and their use as injectable gels for the delivery of therapeutic agents is presented. The technique allows the coupling of a wide range of hydrophilic and hydrophobic amines to hyaluronan carboxylic acid groups by a reaction performed in aqueous media under mild conditions. It involves the use of 2-chloro-4,6-dimethoxy-1,3,5-triazine as an activating species. The key feature is that the number of hyaluronan repeat units that are modified is easily controlled by the amount of triazine that is added. The described modification technique is suitable for introducing a number of functionalities to hyaluronan and can be applied for the design of hyaluronan-based biomaterials including injectable gels for tissue engineering. Using the triazine-activated amidation, an *in situ* cross-linkable hyaluronan derivative was prepared which was evaluated *in vitro* and *in vivo*. The injectable gel was loaded with biologically active proteins and examined *in vitro* by studying release kinetics and *in vivo* by implantation in rats. Release kinetics study revealed a significant sustained cumulative release of the active substance during one week and histological examinations four weeks after the implantations showed a desired tissue response with no sign of

inflammation. We believe that the injectable gel has potential in clinical use as a vehicle for the delivery of therapeutic agents.

(339) Process Capability for Regenerative Medicine: A Preliminary Study of Scaffold Mould Fabrication Process

Liu C., Hourd P., Chandra A., Williams D.J., Czernuszka J.T.

The Wolfson School of Mechanical & Manufacturing Engineering, Loughborough University, Loughborough LE11 3TU, United Kingdom

Department of Materials, University of Oxford, Oxford OX1 3PH, United Kingdom

The sacrifice negative scaffold moulds were created using a commercial solid free fabrication system T66 (Solidscape Inc.). The process capability of the scaffold mould was assessed by using printing deviation in both horizontal and vertical orientations. The process capability indices (Cpk, Ppk and Cpm) were calculated to provide a numerical summary that compares the behaviour of printing process to the engineering specification.

This preliminary work has determined the capability of the scaffold mould process using an indirect 3D fabrication process. It was observed that the scaffold mould printing process is statistical control, and the dimension deviations are normally distributed. The process under normal cooling condition has low Cpk, Ppk and Cpm values of 0.45, 0.55, and 0.4, respectively. Indicated the process under normal cooling condition is not a capable process to manufacture scaffold mould with required dimension specification. However, printing process capability improved greatly under improved cooling condition, with moderate Cpk and Ppk values of 1.56 and 1.66, respectively. Such process approximately fabricate only 2.4 in 1 million printed features fall outside the specification limit, indicate a satisfactory process to manufacture scaffold moulds within the specifications.

The results highlighted the necessary for regenerative medicine engineering to the optimise and improve the process that take account of the regulatory environment.

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(340) Progenitor-Derived Endothelial Cell Therapy Restores Erectile Function

Rodríguez S., Chen K.L., Atala A., Yoo J.J.

Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, United States of America

Introduction: Approximately 35–75% of men with diabetes mellitus are afflicted with erectile dysfunction, which is believed to involve impairment in endothelial cell function. We examined whether endothelial cell therapy could restore normal erectile function in a diabetic rat model. We investigated the feasibility of using endothelial progenitor cells (EPC), obtained from peripheral blood, as a source of endothelial cells for therapy.

Materials and Methods: EPC, isolated from peripheral blood of donor rats, were induced into an endothelial cell lineage. The culture expanded endothelial cells were labeled with red fluorescent dye and injected directly into the dysfunctional corpora of diabetic rats, and followed for up to 12 weeks. Erectile function was assessed by intracavernous pressure and the corporal tissues were retrieved for analyses.

Results and Discussion: The progenitor and differentiated endothelial cell phenotypes were confirmed with cell specific antibodies. The animals injected with cells showed a substantial improvement in intracavernous pressure and their erectile function was restored to normal levels. Histologically, the implanted cells that were labeled with the fluorescent dye tracer PKH26 survived and integrated into the corporal tissue within the injected region.

Conclusions: This study demonstrates that the EPC-derived endothelial cells are able to restore normal erectile function in a diabetic rat model. This cell-based technology may be a viable treatment modality for diabetic patients with erectile dysfunction.

(341) Protein Kinase A Exhibits Both Paracrine and Cell Autonomous Control of Bone Formation by Human Mesenchymal Stem Cells

Siddappa R., Van Blitterswijk C., Gaspar C., Fodde R., Olivo C., Martens A., De Boer J.

Department of Tissue Regeneration, Institute for Biomedical Technology, P.O. Box 217, Enschede, The Netherlands

Human mesenchymal stem cells (hMSCs) are multipotent cells which are increasingly exploited in the field of regenerative medicine and tissue engineering. To further our knowledge on osteogenic pathways, we studied the effect of Protein Kinase A (PKA) activation on osteogenic differentiation of hMSCs. PKA activation using cAMP or cholera toxin strongly enhanced osteogenic differentiation of hMSCs. Microarray and qPCR studies revealed that PKA activation induced cycloheximide-insensitive expression of BMP target genes ID2, ID4 and SMAD6 within 6 hours, demonstrating that PKA directly induces BMP target genes. Furthermore, delayed peak expression of another set of BMP target genes after 5 days of cAMP treatment implied a second, paracrine mode of activation. Indeed, co-incubation of hMSCs with cAMP and noggin, a known inhibitor of BMP signaling, confirmed a paracrine effect of BMPs on target gene expression. In line with this, BMP2 expression is upregulated in cAMP treated cells. PKA activation further induced the expression of osteogenic cytokines and growth factors such as IL-11, IGF1 and other TGF- β family members. As a consequence, PKA strongly enhances the bone forming capacity of hMSCs in immune-deficient mice *in vivo*. We conclude that PKA displays a functional interaction with the BMP signaling pathway at two different levels: directly by activating BMP target genes and indirectly by activating BMP expression. These results provide an important insight into the molecular pathway downstream of PKA signaling and its interaction with other signaling pathways in enhancing osteogenesis *in vitro* and *in vivo*, which can be further applied in the field of regenerative medicine and bone tissue engineering.

(342) Protein Kinase C Epsilon Is Required for Cutaneous Wound Closure and Myofibroblast Formation

Leask A., Shi-Wen X., Black C.M., Abraham D.J.

University of Western Ontario, CIHR Group in Skeletal Development and Remodeling, London, ON, N6A 5C1 Canada
UCL (Royal Free Campus), Department of Rheumatology, London, UK NW3 2PF, United Kingdom

Cutaneous wound repair requires the de novo induction of a specialized form of fibroblast, the alpha-smooth muscle actin (alpha-SMA)-expressing myofibroblast, which migrates into the wound, adheres to and contracts extracellular matrix (ECM) resulting in wound closure. After normal tissue repair, the myofibroblast disappears; however, should the myofibroblast persist, scarring results. Excessive scarring produces fibrotic diseases characterized by organ failure and death. In this report, we show that mice homozygous for a deletion in the PKCepsilon gene, although otherwise appearing phenotypically normal, display markedly delayed cutaneous wound closure and failure to induce myofibroblasts in response to wounding. Isolated PKCepsilon^{-/-} dermal fibroblasts express alpha-smooth muscle actin (alpha-SMA), but are not able to form an alpha-SMA stress fiber network but possess a normal actin filament network, and show significantly reduced focal adhesion (FA) formation, and FAK and rac activation both basally and in response to TGFbeta. Transient over-expression of constitutively active rac in PKCepsilon^{-/-} fibroblasts rescues the defective FAK phosphorylation and cell migration phenotype of these cells. For the first time we identify a kinase selectively required for myofibroblast function. PKCepsilon antagonists would therefore be expected blocking pathological fibrosis. Conversely, PKCepsilon agonists may promote wound healing and regeneration.

(343) Protocatechuic Acid Promotes Cell Proliferation and Reduces Basal Apoptosis in Cultured Neural Stem Cells

Guan S., Ge D., Liu T.Q., Li X.Q., Song K.D., Bao C.Y., Ma X.H., Cui Z.F.

Stem Cell and Tissue Engineering Laboratory, Dalian University of Technology, Dalian 116024, China
Oxford Centre for Tissue Engineering and Bioprocessing, Department of Engineering Science, Oxford University, Oxford OX1 3PJ, United Kingdom

Protocatechuic acid (PCA), a phenolic compound isolated from the kernels of *Alpinia (A.) oxyphylla*, showed antioxidant neuroprotective property in our previous study [1, 2]. However, it is still unknown whether PCA has effects on the development of the central nervous system. In this study, we investigated the effects of PCA on neural stem cell (NSC) proliferation and apoptosis. NSCs obtained from 13.5-day-old rat embryos were propagated as neurospheres and cultured under differential conditions with or without PCA for 4 and 7 days. The cell viability was determined by CCK-8 test, while cell proliferation was assayed by BrdU labeling. PCA significantly increased the cellular viability of NSCs and stimulated cell proliferation. Apoptotic cells were detected after 4 days by observing the nuclear morphological changes and flow cytometric analysis. Compared with the control on both culture

days, treatment with PCA effectively reduced basal levels of apoptosis of NSCs. At the same time, the reactive oxygen species level (ROS) in NSCs was depressed. In addition, PCA also significantly decreased the activity of elevated caspase-3, indicating that PCA may inhibit apoptosis of NSCs via suppression of the caspase cascade. These results suggest that PCA may be a powerful growth inducer and apoptosis inhibitor for NSCs.

References

1. An L.J., Guan S., Shi G.F., et al.(2006) Protocatechuic acid from *Alpinia oxyphylla* against MPP+-induced neurotoxicity in PC12 cells. *Food Chem. Toxicol.* 44, 436–443.
2. Guan S., Bao Y.M., Jiang B., et al.(2006) Protective effect of protocatechuic acid from *Alpinia oxyphylla* on hydrogen peroxide-induced oxidative PC12 cell death. *Eur. J. Pharmacol.* 538, 73–79.

(344) Purifications as an Alternative for Decellularizations in Small-Diameter Blood Vessel Tissue Engineering

Koens M., Fleuren E., Wismans R., Daamen W., Van Kuppevelt T.

Department of Biochemistry, 280, NCMLS, Radboud University Nijmegen Medical Centre, P.O. Box 9101, Nijmegen 6500 HB, The Netherlands

Background: A wide variety of detergent-based decellularizations have been investigated in obtaining a vascular graft for small-diameter blood vessel tissue engineering. However, the chemical effect upon the structure and presence of extracellular matrix (ECM) components constituting the graft—e.g. collagen, elastin and glycosaminoglycans (GAGs)—has lacked attention. On the other hand, purification methods are developed to obtain purified ECM proteins which can serve as building blocks for molecularly-defined grafts.

Methods: Two decellularizations were used; one used CHAPS followed by SDS, another employed Triton X-100. The collagen purification procedure was based on extractions with diluted acetic acid, salt and organic solvents. Two elastin purifications were used; no. 1 consisted of extractions with salt, CNBr/formic acid, and 2-mercaptoethanol and a trypsin digestion, while no. 2 consisted of a single CNBr/formic acid step with subsequent boiling.

Results: Both decellularizations and the collagen purification did not result in complete cell removal from the arteries and displayed a variety of ECM proteins. Elastin purification no. 1 resulted in highly purified insoluble elastin fibres, while no. 2 displayed seriously affected and impure elastic fibres which had lost its tubular shape distally.

Discussion: One should be careful with applying decellularizations, as remaining cellular antigens can evoke xenograft rejection or inflammatory responses. The collagen and elastin purification no. 2 did not result in a desired end product. Only arteries treated with elastin purification no. 1 resulted in a highly purified product with elastin fibres.

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(345) Quality Control of Human Cornea Cell Cultures and Bioengineered Corneal Constructs by Gene Expression Analysis

Alaminos M., Gonzalez-Andrades M., Muñoz-Avila J.I., Garzon I., Serrano D., Medialdea S., Campos A.

Department of Histology (Group of Tissue Engineering), University of Granada, Spain

Division of Ophthalmology, University Hospitals San Cecilio and Virgen de las Nieves, Granada, Spain

Introduction: Establishing quality controls of bioengineered tissues and organs intended for clinical use is an important subject of tissue engineering. In this work, we have determined the global gene expression profile of both primary cultures of human corneal cells and artificial corneas developed by tissue engineering.

Materials and Methods: Primary cultures of corneal epithelial cells and keratocytes were obtained using small explants of corneal limbus from human donors. Cornea constructs were developed using fibrin-agarose scaffolds and air-liquid culture techniques (1). Total RNA from primary cell cultures and cornea constructs was isolated and hybridized to Affymetrix U133-plus 2.0 microarray chips. SAM analytical algorithms were used to identify differentially expressed genes.

Results and Discussion: Comprehensive gene expression analysis of primary cultures and bioengineered corneas demonstrated that primary epithelial cell cultures expressed a wide range of genes related to cell adhesion (desmoplakine, occludine, plakophilin), cytoskeletal proteins (ARPC4) and ectodermic development (KRT8B1, MDK, BTC), whereas corneal constructs expressed high amounts of RNA corresponding to genes associated to basal membrane (laminins), extracellular matrix (lumican, perlecan), angiogenesis (ANGPT1) and development (RUNX2, TBX1, SERPINF1, PTEN, NOTCH2). All these data reveal that corneal epithelial cells tended to progressively mature and stratify, forming a genetically functional barrier on top of bioengineered corneal constructs.

Reference

1. Alaminos et al. (2006). Construction of a complete rabbit cornea substitute using a fibrin-agarose scaffold. *Invest Ophthalmol Vis Sci.* 47:3311–3317.

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(346) Quantification of Neovascularisation in Tissue Engineered Constructs Using Micro-CT

Bolland B.J., Kanczler J.M., Dunlop J., Oreffo R.O.C.

Bone & Joint Research Group, Experimental Origins of Health and Disease, University of Southampton, Southampton, SO16 6YD, United Kingdom

Quantification and ...alization of new vessel networks remains a problem in tissue engineered constructs. A novel technique utilizing a radio-opaque dye and Micro-Computer Tomography

Abstract Withdrawn

(μ CT) has been developed and applied to study angiogenesis in an *in vivo* impaction bone graft model. Tissue-engineered constructs combining human bone marrow stromal cells (HBMSC) with natural allograft and synthetic grafts (Poly D,L-lactic acid, PLA) were impacted and implanted into the subcutis of MF1 nude mice. Radiopaque dye was infused into all vessels via cardiac cannulation prior to removal of implants. Micro CT imaging and immunohistochemistry was performed in all samples.

Cell survival and viability was confirmed using cell tracker green and ethidium homodimer-1. The average number of blood vessels penetrating the capsules were 16.33 in the allograft/HBMSC constructs compared to 3.5 ($p=0.001$) in the allograft alone samples and 32.67 in the PLA/HBMSC constructs compared to 7.67 ($p=0.001$) in the PLA alone samples. The average total vessel volume within the capsules was 0.43 mm^3 in the allograft/HBMSC constructs compared to 0.04 mm^3 ($p=0.05$) in the allograft alone samples and 1.19 mm^3 in the PLA/HBMSC constructs compared to 0.12 mm^3 ($p=0.004$) in the PLA alone samples. Extensive staining for Type 1 Collagen, new matrix and Von Willebrand factor in living tissue engineered constructs confirmed osteogenic cell phenotype, and new blood vessel formation respectively.

This novel technique has been used to demonstrate neovascularisation and bone formation in impacted tissue engineered constructs providing a facile approach with wide experimental application.

(347) Real Time Detection of Stress in 3D Tissue Engineered Constructs Using NF- κ B Activation in Transiently Transfected Human Dermal Fibroblasts

Cantón I., Sarwar U., Kemp E.H., Watson P.F., Ryan A.J., MacNeil S., Haycock J.W.

Department of Engineering Materials, Kroto Research Institute, Division of Clinical Sciences (North) and Department of Chemistry, University of Sheffield, United Kingdom

The European Commission wants to see alternative methods for replacing animals traditionally used for irritation, corrosivity and phototoxicity testing by 2009. We therefore report on the development of a non-destructive reporter system for assessing the response of human skin cells within a 3D tissue engineered construct to exogenous stress. Dermal fibroblasts were transiently transfected with an NF- κ B linked reporter construct which led to expression of a non-stable form of enhanced GFP after stimulation. This caused a detectable production, but was not intrinsically toxic, as cells could metabolise the initial GFP produced. This enabled the model to be used for re-stimulation post recovery. To investigate the method for assessing cellular response to stress in 3D we used a range of compounds known to have pro-inflammatory or oxidative properties. TNF- α , IL-1 β , LPS or H_2O_2 were used to stimulate transfected cells in 3D and the synthesis of GFP detected as a measure of NF- κ B activation. All agents activated NF- κ B when cells were grown in 3D scaffolds but did not cause any significant reduction in cell viability as measured by a standard MTT-ESTA viability test. Parallel NF- κ B activation and MTT measurements were also conducted in 2D and confirmed findings in 3D. The 3D model described using a fluorescent reporter gene is a highly sensitive and reliable method for detecting cellular stress and represents a key step in developing tissue engineering models with the potential for screening pharmaceutical and cosmetic compounds, as an alternative to existing *in vitro* and *in vivo* methods.

(348) Reconstruction of Injured Skeletal Muscle Needs a Balance Between Regeneration and Fibrosis

Li Y., Huard J.

Department of Orthopaedic Surgery and Pathology, University of Pittsburgh; Stem Cell Research Center (S.C.R.C) at Children's Hospital of Pittsburgh, United States of America

Muscle injuries are common in professional and recreational sports. The best treatment for muscle injuries is not yet known, and recommended treatment regimens vary widely on the basis of injury severity. An injured muscle is able to repair itself through muscle regeneration via activation of satellite cells. However, the injury often stimulates overgrowth of the extracellular matrix (ECM) and leads to a local fibrosis. This fibrous scar impedes the formation of normal muscle fibers, resulting in incomplete functional recovery and a propensity for re-injury. We have begun to study the mechanism behind fibrosis occurring in injured skeletal muscle. Our studies have demonstrated that myogenic cells and regenerating myofibers in lacerated muscle can differentiate into fibrotic cells, and that TGF- β 1 is a key factor in the activation of the fibrosis cascade in skeletal muscle. Thus this molecule has become a key target for our antifibrotic therapies. By using different antifibrotic agents to minimize the effects of TGF- β 1 in various animal models of muscle injury, we have both prevented fibrosis and improved muscle healing as assessed histologically and physiologically. Therefore, there is a persistent imbalance between collagen biosynthesis and degradation contributes to hypertrophic scar formation and fibrosis in cases of traumatic injury or disease. In light of the circumstances that usually surround the development of fibrous scar tissue, the development of a way to prevent scar tissue would be of great clinical relevance.

(349) Regenerative Potential of Silk Conduits Following Peripheral Nerve Injury

Huang W.L.¹, Begum R.¹, Yang Q.¹, Gheysens T.², Skaer N.², King V.¹, Knight D.², Priestley J.V.¹

¹Neuroscience Centre, Institute of Cell and Molecular Science, Queen Mary University of London, London, E1 2AT, United Kingdom

²Oxford Biomaterials Ltd., Newbury, RG19 6HR, United Kingdom

Peripheral nerve injury (PNI) leads to sensation and movement loss that is very debilitating for the patient. Attempts using artificial conduits have been made to encourage nerve repair, but the success is still limited. Here, we have developed and tested conduits made of Spidrex, a novel silk-based biomaterial with properties similar to those of spider silk.

Studies in culture showed that disassociated dorsal root ganglion neurons extend long neurites along Spidrex fibers and Schwann cells adhere to the fibers. Conduits were fabricated comprising a silk outer wall and a lumen contained longitudinally aligned silk fibers. For assessment *in vivo*, a segment of the left rat sciatic nerve was removed and a 1 cm silk conduit was implanted. As controls, separate experiments were set up in which a 1 cm segment of sciatic nerve was removed and then re-implanted as an autologous graft. At 4 weeks, animals were perfused and then the implanted conduit along with 1 cm distal nerve were removed and processed for immunocytochemistry. Transverse and longitudinal sections

revealed numerous immunoreactive axons. For example, conduits containing 200 luminal fibres showed 70% of axonal growth at the mid-conduit level as compared to an autograft graft, and 59% of growth at the 1-cm distal nerve level compared to an autograft graft. The distal nerve showed similar macrophage and Schwann cell responses to the conduits as to the autologous graft. Our data indicate that Spidrex conduits support excellent regeneration and have potential for use in treatment of PNI.

(350) Regulation of Human Dermal Fibroblasts and Bone Marrow Stem Cells Proliferation by the Fibronectin/Fibrin Ratio in Gels

Alovskaya A., Alekseeva T., Brown R.A.

UCL, Tissue Repair and Engineering Centre, Institute of Orthopaedics, Stanmore, London, HA7 4LP, United Kingdom

Fibrin is an extracellular (ECM) protein associated with early stage tissue repair and is used widely as glue in many surgical procedures. However its role in tissue engineering has restricted its ability to support integrin mediated cell attachment and functional regeneration. We have previously shown that it acts as a good scaffold for neural regeneration when gelled with fibronectin. In the current study we have tested fibronectin rich fibrin gels (fn/fb) for proliferation of different cell types: human dermal fibroblasts (HDF) and bone marrow stem cells (BMS).

Fibrin gels (1 ml per well) were made by mixing components: 5.5 mM Ca^{2+} and 2 NIH units/ml of thrombin with various ratios of fibrinogen (fb) and fibronectin (fn). Cell viability was assessed by fluorescence with using live/dead staining Calcein AM—Ethidium homodimer-1 and cell proliferation was estimated by alamar-blue assay.

During the 7 days of culture both cell types proliferated rapidly in fibrin gels containing from 10% to 50% of fibronectin. HDF and BMS took an elongate shape up to 6–8 hours after seeding. Cell viability was 80–85% in both fn/fb and fb gels. Addition of exogenous fibronectin enhanced proliferation of HDF and BMS. Increased division was dependent from ratio of fibronectin/fibrinogen.

It is well known that ECM integrin-mediated cell adhesion provides signals essential for cell cycle progression and it seems likely that the simple incorporation of additional level of fibronectin into fibrin gels will dramatically improve cell growth and maintenance dense cell population deep in 3D.

(351) Regulation of MMP's in Endothelial Cell-Seeded UBM Extracellular Matrix Under Shear

Callanan A.¹, Morris L.², McGloughlin T.¹, Gilbert T.W.³, Badylak S.F.³

¹Dept. Mechanical and Aeronautical Engineering, Centre for Biomedical Engineering Research, (CABER) University of Limerick and MSSl, Limerick, Ireland

²Dept. Mechanical and Industrial Engineering, Galway Mayo Institute of Technology, Galway, Ireland

³McGowan Institute for Regenerative Medicine, University of Pittsburgh, United States of America

Cardiovascular diseases are still the leading cause of death in Western society. Most cardiovascular diseases are treated with arterial

substitutes such as synthetic grafts. These result in increased wave-reflection loading on the patient's heart. In recent years a change toward tissue engineered vascular grafts has been examined to address this inadequacy. The Development of large diameter tissue engineering grafts would result in better remodeling capabilities, which may reduce the compliance mismatch and decreased heart loading.

This study examines the role of matrix metalloproteinase (MMP) gene expression in human aortic endothelial cells (HAEC's) when attached to Urinary Bladder Membrane (UBM), an extracellular matrix (ECM) as a large diameter graft. The ECM material is mainly collagen-based and is derived from porcine urinary bladder. This ECM material was constructed into a 4-ply 20 mm diameter vascular construct/graft using a vacuum technique. The ECM tubes were seeded with human aortic endothelial cells (HAEC) at a density of $0.4 \times 10^6/\text{cm}^2$ using a novel roller bottle system. The seeded tubes were then placed into the bioreactor flow chamber and a steady shear flow was applied for 24 hours.

The ECM tubes showed high cell attachment and proliferation on the ECM surface using a live/dead assay after the flow tests. Gene expression was measured using real time RT-PCR. The MMP's gene expression was up-regulated after the application of the steady flow, indicating the possible initiation of endothelial cell remodeling of the UBM collagen based material as a result of the applied steady shear flow.

(352) Regulation of Oxygen and pH in a Bioreactor for Cartilage Tissue Engineering

Das R., Kreukniet M., Jahr H., Van Osch G., Weinans H.

Erasmus MC, University Medical Center, Dept orthopaedics, Rotterdam, The Netherlands

Introduction: Control of environmental parameters like oxygen tension and pH can be employed to improve the quality of cartilage tissue-engineered constructs. To efficiently investigate the response to different environmental conditions, a system that enables monitoring and feedback control with a reasonable high throughput is desirable. We tested the efficacy of a bioreactor that allows control over dissolved oxygen (DO) and pH for tissue-engineering purposes.

Materials and Methods: A microbioreactor (micro-24, Applikon Biotechnology, Schiedam) was tested for its ability to maintain a chondrocyte culture in a controlled environment. Accuracy and consistency of DO and pH control were evaluated for prolonged operation. Time necessary to reach a set-point state and variation around set-point were determined for three levels of DO and six levels of pH. In addition, sensor drift was established for pH measurements over two weeks.

Results: Set-point was reached in approximately 30–60 minutes and subsequently maintained for 5 days. pH variations remained within 0.1 of set-point, while DO-control accuracy varied with set-point (higher accuracy was achieved at lower DO, with variations less than 2% around set-point at 25% DO). No significant difference was found in pH values obtained from online and offline measurement. Measurements remained consistent over two weeks, indicating no sensor drift occurred.

Conclusion: A novel bioreactor was tested for potential use in tissue engineering applications. Prolonged and accurate control of

DO and pH was found, enabling testing of multiple configurations within one experiment. A pilot experiment confirmed the suitability of this bioreactor for cartilage tissue engineering purposes.

(353) Release of Pro-angiogenic Factors from Adipose Tissue Derived Stromal Cells in Dependency of Differentiation

Peters K., Fiedler T., Salamon A., Adam S., Weber J., Rychly J.

Junior Research Group "Bone Regeneration", Department of Cell Biology, Biomedical Research Centre, Schillingallee 69, 18057 Rostock, Germany

Ästhetik-Klinik Dr. Weber, Am Strande 3, 18055 Rostock, Germany
Department of Cell Biology, Biomedical Research Centre, Schillingallee 69, 18057 Rostock, Germany

Adipose tissue derived stromal cells (ASC) have multilineage differentiation capacity. Therefore, ASC may have the potential to be used for various clinical applications, including bone, cartilage, and cardiac tissue regeneration. An important step during regeneration of tissues is the vascular supply. This vascular supply is induced by pro-angiogenic factors (e.g. vascular endothelial growth factor/VEGF) released by tissues requiring vascularization.

Recent studies revealed a cell population within freshly isolated ASC showing a distinct expression of CD34, a well-known marker of hematopoietic stem cells. These cells were able to perform osteogenic and adipogenic differentiation after specific stimulation. Since the characteristics of this ASC-subpopulation are rather unidentified, we have compared CD34-positive (CD34+) and CD34-negative (CD34-) human ASC regarding the release of the pro-angiogenic factor VEGF in a non-stimulated state and under osteogenic differentiation.

In principle, both ASC-subpopulations performed osteogenic differentiation. However, the degree of osteogenic differentiation and the release of VEGF were higher in CD34- cells. Interestingly, osteogenic stimulation induced a significant downregulation of VEGF release in both ASC-subpopulations (ca. 50% reduction). Thus, both ASC-subpopulations showed differences regarding their osteogenic differentiation potential and in the release of the pro-angiogenic factor VEGF. However, knowledge about the CD34+ and CD34- ASC-subpopulations remains fragmentary and needs further investigation.

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(354) Rendering PET Substrate Bioactive for Endothelial Cell Adhesion by RGD Mimic Grafting

Rerat V., Bareille R., Bordenave L., Marchand-Brynaert J.

Inserm, U577, Bordeaux, F-33076 France; Univ Victor Segalen Bordeaux 2, Bordeaux, F-33076, France

Engineered bioactive polymeric scaffolds are of increasing importance for use in cell culture systems and tissue regeneration. However, polymer surfaces are often prone to nonspecific protein adsorption which can lead to nonspecific cell-type adhesion. Our work concerns the promotion of endothelial cell (EC) adhesion on PET (polyethyleneterephthalate) substrates. For that purpose, we

have exploited the selective "ligand-integrin" interaction. The $\alpha v \beta 3$ integrin subgroup, largely expressed on ECs, recognizes adhesive proteins and peptides including the RGD (Arg-Gly-Asp) sequence in a particular conformation as exemplified with cyclic RGD peptides. We used such molecules as models to establish the design of nonpeptide mimics of the natural adhesive products. Based on the tyrosine template, peptidomimetics of the c[RGDfN(Me)V] peptide have been prepared and equipped with a short PEG spacer-arm allowing their covalent grafting on PET track-etched membranes. One representative peptidomimetic molecule, displaying an IC₅₀ value of 0.3 nM versus human isolated $\alpha v \beta 3$ integrin, was selected for a systematic study of Human Saphenous Vein EC (HSVEC) adhesion on the modified substrate. HSVECs, after seeding at 50,000/cm² on (un)modified membranes in the absence of serum, showed an increasing adhesion rate, as for controls, at 1, 3, and 6 h on grafted surfaces only (80%) using a quantitative colorimetric test. On modified PET, confocal microscopy of vinculin revealed numerous cell focal contacts at 16 h post-seeding. Thus, this approach is valid and promising for *in vitro* EC colonization in vascular tissue engineering. Further works are in progress for studying cell proliferation and response under shear stress.

(355) Reporter-Vector Systems to Monitor Osteogenic Differentiation

Feichtinger G., Kloesch B., Kehrer I., Dopler D., Banerjee A., van Griensven M., Redl H.

Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Centre—Austrian Cluster of Tissue Regeneration—Vienna, Austria

BMPs trigger osteoblastic differentiation of capable mammalian cells through TGF- β -family signaling pathways. The transcription factor Cbfa-1/Osf2 that is initially activated by this signal transduction cascade drives the expression of osteoblastic marker genes like osteocalcin, osteopontin, bone sialoprotein and collagen I through tissue specific cis-acting elements. The bioactivity of recombinantly expressed BMPs and other osteogenic substances for bone tissue engineering applications needs to be assayed *in vivo* and *in vitro*. The employed standard procedures are the ectopic bone formation assay *in vivo* and the alkaline phosphatase assay *in vitro* and *ex vivo*.

Our primary aim was to design and evaluate new reporter-vector systems based on Cbfa-1/Osf2 activated promoters *in vitro* concerning their possible application for BMP bioactivity assays. We recently investigated a transiently transfected reporter-vector based on the murine osteocalcin-2 (mOG-2) promoter linked to the open reading frame of the Enhanced Yellow Fluorescent Protein (EYFP) that showed high specificity but weak reporter gene expression upon stimulation with rhBMP-2 in C2C12 murine myoblasts. Therefore as second aim we evaluated the signal amplification potential of viral enhancers.

The new construct employs a cytomegalovirus enhancer cloned directly upstream of the murine OG2-promoter that drives expression of the reporter gene EYFP. Additionally we designed a murine collagen I $\alpha 1$ reporter-vector. This construct drives osteoblast specific expression of the reporter gene dsRed via collagen I $\alpha 1$ —enhancer and promoter elements. Both vectors were transiently transfected in C2C12 murine myoblasts and rhBMP-2 induced reporter gene expression was detected by fluorescence microscopy.

Marker gene and reporter gene expression monitoring (e.g. osteocalcin, BSP, Cbfa-1/Osf2, osterix, EYFP, dsRed) by RT-PCR and alkaline phosphatase assays were carried out for a period of 14 days to prove the osteoblast restricted activity of our reporter-vectors. Furthermore, tissue specific activity of the CMV-enhanced construct was confirmed by transfection of the HEPG2 cell line (hepatocytic origin).

C2C12 myoblasts transfected with the CMV-enhanced construct showed strong reporter gene expression upon stimulation with rhBMP-2 (500 ng/ml), and clustering of EYFP positive cells was observed by day 7 and more prominent by day 14. HEPG2 cells transfected with the same construct exhibited weak background expression of the reporter gene confirming its tissue-specificity. The collagen I $\alpha 1$ reporter vector is currently under investigation.

We conclude from our *in vitro* results concerning the CMV-enhanced mOG2-vector that viral enhancers amplify reporter gene expression without altering the behavior of the employed tissue specific promoters. The newly developed CMV-enhanced system allows the real time monitoring of osteoblastic differentiation *in vitro* by fluorescence microscopy therefore providing a sensitive bioactivity assay for osteogenic substances in C2C12 cells.

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(356) Rheological Properties of a Fibrin-Agarose Corneal Substitute

Gomez-Sotomayor R., Alaminos M., Duran J.D.G., Garzon I., Fernandez-Montoya A., Delgado C., Campos A.

Departments of Histology (Group of Tissue Engineering) and Applied Physics, University of Granada, Spain
University Hospital San Cecilio, Granada, Spain
CRTS and Tissue Bank of Granada, Spain

Introduction: In recent years, new biomaterials have been used for the construction of artificial corneas by tissue engineering (1). However, a good biomaterial should have several mechanical properties such as viscosimetry and oscillometry. In this work, we have analysed the rheological properties of a fibrin-agarose corneal substitute developed by tissue engineering.

Materials and Methods: Partial corneal substitutes were developed by culturing stromal keratocytes immersed in fibrin-agarose hydrogels. Rheological analysis of the corneal constructs was carried out in a CS-10 Bohlin rheometer using plate-plate configuration. To determine the eventual modifications that might happen in our biomaterials along the time, we performed the rheological analyses at time 0, 1, 2, 3 and 4 weeks after the development of the corneal constructs. As a control, we used fibrin-agarose hydrogels without the addition of cells.

Results and Discussion: Our analysis revealed that the mechanical properties of the constructs were intrinsically bound to the different biomaterials used, with shear and stress rates depending on the polymerization and the internal molecular bonds of the biomaterials. Viscosimetry and oscillometry rheological analyses showed that the fibrin-agarose constructs tended to display a typical visco-elastic behaviour that is time-related. These results suggest that the

mechanical properties of the corneal substitutes were similar to those of normal, native corneas.

Reference

1. Alaminos et al. (2006). Construction of a complete rabbit cornea substitute using a fibrin-agarose scaffold. *Invest Ophthalmol Vis Sci.* 47:3311–3317.

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(357) Role of Platelet-Derived Growth Factor Receptor Beta in Wound Healing and Tissue Fibrosis

Shiwen X., Rajkumar V., Khan K., Denton C., Chen Y., Eastwood M., Black C., Abraham D.

Department of Rheumatology, University College London, Rowland Hill St., London NW3 2PF, United Kingdom
School of Biosciences, University of Westminster, London, W1W 6UW, United Kingdom

Connective tissues provide mammals with a mechanism to repair wounds after injury. Tissue remodelling is characterised by mesenchymal cell activation, recruitment and differentiation, processes dysregulated in human scarring diseases and fibrosis. Here, we use knock-out fibroblasts and transgenic reporter mice to study disruption and blockade (imatinib mesylate) of platelet-derived growth factor receptor beta activity (PDGFR-beta) on key mesenchymal cell types crucial to wound healing and the fibrogenic activities of dermal and pulmonary fibroblasts derived from patients with the fibrotic disease scleroderma (SSc).

PDGFR-beta $-/-$ fibroblasts exhibited reduced migration, contractile responses and remodeling of 3-dimensional collagen matrices ($p > 0.05$). *In vivo* PDGFR-beta inhibition delayed wound repair, PDGFR-beta signalling being essential for the recruitment, proliferation and functional activities of fibroblasts and mesenchymal pericytes. *In vitro* PDGFR-beta blockade inhibited fibroblast migration and proliferation, but did not inhibit TGF-beta dependent myofibroblast formation. Although treatment of control fibroblasts with imatinib had little effect, SSc fibroblasts were severely impaired in their ability to contract collagen lattices ($p > 0.05$) and exhibited a profound block in migration following *in vitro* scratch wounding. These inhibitory activities were strongly correlated with significantly reduced levels of the phosphorylated forms of p42/p44.

This data highlights the significance of PDGFR-beta signaling in recruitment, proliferation, and functional activities of mesenchymal cells during wound healing, and provide the rationale for PDGFR-beta blockade in fibrotic diseases such as SSc.

(358) Scaffold Density Influences the Synthesis and Retention of Extracellular Matrix by Articular Chondrocytes

Raveenthiran D., Rashid A.T., Wang M-J., Mole C., Bader D.L., Knight M.M.

School of Engineering and Material Science, Queen Mary University of London, Mile End Rd, London, E1 4NS, United Kingdom

One strategy for cartilage tissue engineering is to seed cells within a scaffold enabling the synthesis of extracellular matrix. This study tested the hypothesis that scaffold density influences

micro-porosity, mechanical properties and the synthesis and retention of cartilaginous matrix.

Bovine chondrocytes were seeded at 4×10^6 cells/mL into constructs (5 mmDIA \times 5 mm) prepared with 2%, 3% and 4% (w/v) agarose and cultured in DMEM + 20% FCS. At day 1, construct mechanical properties and micro-porosity were determined using unconfined uniaxial compression and AFM, respectively. At days 1, 3, 9, 15, 22 and 29 the total DNA and GAG contents within constructs and the corresponding cumulative GAG released into the medium were determined using the Hoechst and DMB assays respectively.

Increasing agarose concentration reduced pore size, increased tangent modulus and reduced viscoelastic stress relaxation. Concentration had no effect on cell proliferation. However, cells within 2% agarose synthesised more GAG than those in 4% agarose ($p < 0.05$), although differences between 2% and 3% agarose were not statistically significant. Over the 29 day period, increasing proportions of synthesised GAG were lost to the medium, up to 40–45% of total GAG. Both the amount of GAG retained in the construct and that released to the medium were significantly greater for 2% and 3% agarose compared to 4%.

Reducing scaffold porosity increased mechanical properties but reduced the synthesis of matrix. The mechanism may involve alterations in diffusion of nutrients and matrix molecules and steric inhibition. This provides a challenging dilemma for tissue engineers who need to generate mechanically functional scaffolds whilst maximising matrix synthesis and retention.

(359) Scaffold System Suitable for Implant-Type Tissue-Engineered Cartilage

Tanaka Y., Yamaoka H., Nishizawa S., Ogasawara T., Fujihara Y., Asawa Y., Takato T., Hoshi K.

Department of Cartilage & Bone Regeneration (Fujisoft) and Division of Tissue Engineering, Graduate School of Medicine, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8655, Japan

Tissue-engineered cartilage with autologous chondrocytes is already available for clinical use. However, present applications are confined to the treatment for focal cartilage defects. To broaden the indication range for advanced stage of osteoarthritis or the congenital anomalies, an implant-type tissue-engineered cartilage with greater firmness and a 3D-structure should be made. We attempted to develop a scaffold system to realize 1) sufficient mechanical mimicking native cartilage, 2) preservation of seeded cells and their even distribution, and 3) good biocompatibility/biodegradability, by the combination of hydrogel and porous body. At first, we examined typical hydrogels such as atelocollagen, alginate and PuraMatrix. Considering biological effects and clinical availability, atelocollagen may be accessible for clinical use. Next, we investigated the structure and composition of porous body. The mechanical properties and preservation of cells mainly depend on its structure. We prepared ones of a classical polymer PLLA with various kinds of porosity and pore sizes, by sugar-leaching method or fused deposition molding. The PLLA porous body possessed sufficient strength even with high porosity and good interconnectivity, which showed favorable cartilage regeneration when transplanted in the subcutaneous space of nude mice with chondrocyte/atelocollagen mixture. However, ones of excessively large pore

sizes (> 1 mm) broke out on the skin and impaired host tissue if the cell/gel mixture had shrunk. The compositions influenced biocompatibility and biodegradability. We also examined various copolymers of lactide and discussed about cartilage properties and surface smoothness. Such approaches would enable an early clinical application of the implant-type tissue-engineered cartilage.

(360) Selection of Human Mesenchymal Osteoprogenitors from Bone Marrow Stromal Cell Populations Using Polymer Microarrays

Tare R.S., Khan F., Tourniaire G., Morgan S.M., Bradley M., Oreffo R.O.C.

University of Southampton, Bone and Joint Research Group, Southampton General Hospital, Southampton, SO16 6YD, United Kingdom

University of Edinburgh, School of Chemistry, Edinburgh, EH9 3JJ, United Kingdom

Bone marrow stromal cells represent a heterogeneous population, which includes reticular endothelial cells, fibroblasts, adipocytes and osteoprogenitors. One of the approaches for enrichment of osteoprogenitors from human bone marrow mononuclear cell (hBMMC) populations involves immunoselection of these progenitors using the STRO-1 monoclonal antibody. Traditional methods like magnetically-activated cell sorting (MACS) and FACS for immunoselection of STRO-1+ cells are expensive and require skilled personnel. The present study utilised a polymer library comprising of well characterised poly(urethanes) printed in a microarray format on aminoalkylsilane slides for selection of STRO-1+ osteoprogenitors from hBMMC populations. The slides were coated with a thin layer of agarose before contact printing the polymers to minimise nonspecific cell binding. Arrays comprising of 120 poly(urethanes) were incubated overnight with freshly-isolated (using the MACS technique) STRO-1+ cells labelled with CellTracker Green. Analysis using a fluorescent microscope that allowed automated capture of images for each polymer spot and the Pathfinder™ software identified 31 poly(urethanes) binding specifically to STRO-1+ cells. When focused arrays comprising of these 31 poly(urethanes) were incubated overnight with hBMMC samples, in which the osteoprogenitors were immunolabelled with the STRO-1 antibody bound to a FITC-tagged secondary antibody, 4 poly(urethanes) were able to selectively immobilise the STRO-1+ osteoprogenitors from the hBMMC populations. These 4 poly(urethanes) were highly specific for osteoprogenitors from hBMMC as they exhibited very low affinities for binding to chondrogenic ATDC5, myogenic C2C12 and osteoblast-like MG63 cells. This novel platform therefore serves as an important tool in applications where selective isolation of mesenchymal progenitor populations is desired.

(361) Self-renewal of Non-adherent Bone Marrow Clonogenic Progenitors *In Vitro*

Di Maggio N., Banfi A., Schaeren S., Heberer M., Martin I.

Departments of Surgery and of Research, Basel University Hospital, Basel, Switzerland

Bone marrow stromal cells (BMSCs) rapidly lose their differentiation potential during *in vitro* culture. Therefore it is desirable to maximize the yield of clonogenic cells in order to limit their expansion. In this regard, we investigated whether the non-adherent fraction of BM cells, which is normally discarded after three days of culture, contains CFU-f and, if so, how their proliferative and differentiation potential compares to early-adhering BMSCs.

We found that not only was a non-adherent clonogenic population always present, but it could be maintained and even increased over at least 4 serial replating steps (replate 1 = $17.5 \pm 3.0\%$, replate 2 = $44.9 \pm 14.5\%$, replate 3 = $72.3 \pm 21.7\%$, replate 4 = $109.4 \pm 34.5\%$ of the number of colonies generated in the primary plate). The colonies produced in the first, second and third replating were significantly larger than the primary colonies (replate 1 = 10.7 ± 2.7 mm, replate 2 = 8.8 ± 1.5 mm and replate 3 = 7.5 ± 0.7 mm vs. the primary plate = 5.5 ± 1.0 mm diameter, t-test $p < 0.02$). Furthermore, the progeny of non-adherent progenitors replated after three days proliferated faster (15.7 ± 1.2 vs 11.8 ± 1.2 doublings in 14 days) and differentiated better in multiple lineages compared to early-adherent population.

Interestingly this phenomenon could only be observed in the presence of FGF-2, suggesting that this factor has a role in maintaining and regenerating the clonogenicity of this class of progenitors.

In conclusion, these results indicate that non-adherent clonogenic cells exist in BM stroma, which can originate adherent colonies while regenerating themselves and maintaining their clonogenic potential, over several rounds of serial replating and under the control of FGF-2, suggesting a self-renewal step.

(362) Sex-Steroids Influencing Chondrogenesis of Human Mesenchymal Stem Cells (hMSCs)

Jenei-Lanzl Zs., Dienstknecht T., Kujat R., Nerlich M., Angele P.

University Regensburg Clinic, Department of Trauma Surgery, Franz-Josef-Strauss-Allee 11, D-93053 Regensburg, Germany

Introduction: Limited regeneration capacity of defective cartilage represents a serious clinical problem. Tissue engineering using autologous MSCs provides an ideal option for cartilage replacement. In different studies the influence of sex-steroids on cartilage has been demonstrated. We hypothesized that chondrogenic differentiation of hMSCs could be modulated by steroid-hormones.

Methods: Bone marrow derived hMSCs were cultured as cellular monolayer in serum-containing medium. After achieving confluence, the resulting aggregates were cultured under serum-free conditions. Dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT) and 17 β -estradiol (E2) were added in differential concentrations as medium substrates. After 21 days we examined the aggregates macroscopically, by histology and immunohistology. Type II collagen expression (RT-PCR, ELISA), DNA and glycosaminoglycan (GAG) concentrations were measured. Statistical analysis was performed using a two-way ANOVA ($p < 0.05$).

Results: Chondrogenesis was detected in all aggregates. Smaller aggregates were found to result from addition of E2, compared to DHEA, DHT and standard condition. Similar, the collagen type II and GAG content was significant lower for E2, whereas DHEA and

DHT showed effects comparable to the standard culture. The DNA content was constant in all groups.

Discussion: We found that *in vitro* chondrogenic differentiation of hMSCs can be modulated by sex-steroids. Addition of E2 showed inhibitory effects in contrast to DHEA and DHT. Assessing the detailed mechanisms is focus of our current and future work.

(363) Shunting Between Severed Artery and Vein Stumps: The Cause or Result of Neovascularization?

Demirtas Y., Engin M.S., Yagmur Ç., Gurgor P.

Ondokuz Mayıs University Medical School, Departments of Plastic, Reconstructive and Aesthetic Surgery, and Histology, Turkey

Introduction: A good deal of effort is put in the studies to unravel the mechanisms of angiogenesis. In this paper, we are presenting our remarks regarding this topic.

Materials and Methods: As a group predominantly engaged in neoangiogenesis studies, we noticed that, in some specimens, arteriograms of the main pedicles displayed a double rail appearance. Staining of veins was unexpected on those specimens since the solution we have injected was not able to pass to the venous system through the capillary network. Thus, the specimens were dissected under the operating microscope under high magnification.

Results: The micro dissections confirmed the second shadow coursing along the arteries to be veins. The degree of staining was predominantly higher in pedicles in which abundant angiogenesis was achieved.

Conclusion: The magnitude of shunting seems to correlate with the magnitude of angiogenic response. We believe that further dwelling into these findings is likely to shed some light to possible mechanisms of this process. Studies designed deliberately for this topic is under way.

(364) Signalling Between Chondrocytes from Different Zones in Cartilage

Stoddart M.J., Chen Y., Alini M.

AO Research Institute, Biomaterials and Tissue Engineering, Clavadelstrasse 8, Davos Platz, Switzerland

Cartilage is composed of three zones, superficial, middle and deep, which are distinguishable microscopically. Although chondrocytes are considered a single cell type, it is known that the cells from the different zones behave differently when cultured individually. This study compared the matrix synthesis of chondrocytes grown from the individual zones to the mixed population of chondrocytes isolated from full thickness.

Bovine chondrocytes were isolated from full thickness knee joints. Either the individual zones were dissected and then digested or cells were isolated from full thickness cartilage. The cells were resuspended in fibrin and 10 million cells/scaffold seeded in biodegradable polyurethane scaffolds (8 mm by 4 mm). The constructs were then cultured in DMEM (low glucose) + 10% FCS

for 2 weeks. Glycosaminoglycan (GAG), DNA and mRNA were quantified.

Cells from the superficial zone produced very little GAG/DNA, and this was also reflected in the mRNA analysis. Cells from the middle and deep zones both produced similar amounts of GAG/DNA and in both cases GAG production was greater than superficial cells. The full thickness mixed population however demonstrated a GAG synthesis more similar to that of superficial chondrocytes, with very low GAG/DNA.

While the cells from the different zones behaved as expected, the mixed population did not result in an average expression, but instead behaved more similarly to the superficial zone cells. This is likely to be due to some form of signalling between the cells. Whether this is cell-cell or paracrine signalling is unclear but work is underway to elucidate the mechanisms.

(365) Simulation of Dynamic Growth of Neurosphere with Cellular Automata

Liu T.Q., Ju Z.H., Ma X.H., Cui Z.F.

Dalian R&D Center for Stem Cell & Tissue Engineering, Dalian University of Technology, China

The treatment or transplantation strategy of neurodegenerative disorders needs a large amount of neural stem cells (NSCs) because of their potential to be an unrestricted source of cells used in the research and clinical practice.

These cells can be obtained by culturing the NSCs under high-density *in vitro*. One major culture style is three dimensional neurosphere cultures. When the size of a neurosphere cultured *in vitro* reaches a certain critical value, a necrotic core will appear inside the neurosphere because of the limitation of oxygen or other nutrients in the neurosphere. Therefore, it is important to simulate the dynamic process of the growth of neurospheres and analyze the critical size of the neurosphere at which the necrotic core appears. A cellular automata (CA) approach is proposed in this study to model the growth of NSCs in sphere state. The appearance and enlargement of the necrosis in a neurosphere is calculated by coupling the CA model with the nutrient diffusion analysis. The results indicate that the culture conditions, such as seeding density, the concentration of nutrients in medium and the mass transfer coefficient between a neurosphere and medium have some effects on the appearance of the necrosis. However, the necrotic core mainly depends on the inner diffusion. The size of the sphere is hence more critical. Oxygen diffusion limits the viability inside the neurosphere more than other nutrients such as glucose. The growth of the necrotic core is very fast after its appearance, and the whole neurosphere may become necrotic.

(366) Simvastatin Releasing Novel PCL Scaffolds in Rat Cranium Defects

Pişkin E.¹, Isoglu A.¹, Bolgen N.¹, Griffiths S.⁴, Vargel I.², Cavusoglu T.², Korkusuz P.³, Guzel E.³, Cartmell S.⁴

¹Hacettepe University, Chemical Engineering Department and Bioengineering Division, Beytepe, 06800 Ankara, Turkey

²Kırıkkale University, Faculty of Medicine, Department of Plastic and Reconstructive Surgery, Kırıkkale, Turkey

³Hacettepe University, Faculty of Medicine, Department of Histology and Embryology, Ankara, Turkey

⁴Keele University, Institute for Science and Technology in Medicine, Staffordshire, United Kingdom

The objective of this study was to evaluate reconstruction of critical-size calvarial bone defects using a simvastatin releasing novel biodegradable/electrospun/spiral-wounded poly(ϵ -caprolactone) (PCL) scaffold. Nonwoven films composed of nanofibers were prepared by electrospinning from the blends of high and low molecular weight PCLs. Simvastatin was loaded during electrospinning to the matrices in order to include osteoblast activity. The matrices were then spiral-wounded to reach 3D-structures. Rats were selected as model animals, and 8-mm-diameter cranial defects were created. Scaffolds with or without simvastatin were implanted into these critical size 8-mm-diameter cranial defects (no-self-healing size). Samples from the implant sites were removed after 1, 3 and 6 months postimplantation. Bone regeneration and the amount and mineralization of newly formed bone were evaluated by using x-ray microcomputed tomography (microCT) analysis. Tissue response and new tissue formation which takes place in the implant sites were analyzed by histological tests. The *in vivo* results exhibited that osseous tissue integration within the implant and functionally stable restoration of the calvarium. In the time frame, tissue formation was started in the macrochannels in the novel spiral-wounded scaffolds as expected. Both microCT and histological data clearly demonstrated the positive (enhanced) healing of the defects in which the simvastatin releasing scaffolds were used.

(367) Solid Free-form Fabrication of Nerve Guide Conduits for Developing a 3D *In Vitro* Neurogenesis Model

Van Vlimmeren M.¹, Mackay-Sim A.², Woodfield T.³

¹Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands

²Eskitis Institute for Cell and Molecular Therapies, School of Biomedical & Biomedical Science, Griffith University, Brisbane, Australia

³Centre for Bioengineering, University of Canterbury & Department of Orthopaedic Surgery, Christchurch School of Medicine, New Zealand

Promising alternative cell sources for stimulating spinal cord injury (SCI) repair include cells isolated from the olfactory mucosa, namely olfactory ensheathing cells (OECs) [1, 2]. Few studies have focused on using OECs to stimulate axonal growth in nerve guide conduits, or used solid free-form fabrication (SFF) techniques to improve the ability to control axon growth and orientation [3].

Our objectives were to (1) develop advanced nerve guide conduits with optimised channels using SFF to promote high density axonal growth; and (2) develop *in vitro* culture and evaluation techniques for rat OECs and olfactory mucosa-derived neurospheres in a 3D nerve guide model for evaluating axon growth in response to single or multiple cell sources and/or neurotrophic stimuli.

Purity of OEC cultures was evaluated using immunostaining indicating the presence of the glial marker, glial fibrillary acidic protein (GFAP) and the low affinity receptor for nerve growth factor p75NTR.

Using computer aided design (CAD) and a layer-by-layer mask lithography SFF process (EnvisionTec), negative moulds were produced containing axial columns from Ø50–450 µm. Poly(2-hydroxyethyl methacrylate) (pHEMA) gels (60 wt%) were cast in moulds to produce preliminary nerve guides with controlled channel diameter and density. Immunostaining and SEM showed presence of OECs within nerve guide channels.

Alternative substrates as well as axon outgrowth from olfactory neurospheres and olfactory mucosa slice biopsies in channels with or without OECs are currently being evaluated to verify the neurogenesis model.

References

1. Raisman et al. OECs & repair of brain and spinal cord injuries. *Clon Stem Cell*, 2004. 6(4).
2. Lu et al. OECs promote locomotor recovery after delayed transplantation into transected spinal cord. *Brain*, 2002. 125(Pt 1).
3. Moore et al. Multiple-channel scaffolds to promote spinal cord axon regeneration. *Biomater*, 2006. 27(3).

(368) Static and Dynamic Cell Seeding of Bone Composite Scaffolds in a Perfusion Bioreactor

Koch M.A., Engel E., Planell J.A., Lacroix D.

Institute for Bioengineering of Catalonia (IBEC), Universitat Politècnica de Catalunya, Diagonal 647, 08028 Barcelona, Spain

One approach in bone tissue engineering is the use of *in vitro* cell-loaded 3D porous scaffolds. Optimisation of the cell seeding technique is crucial to obtain a high cell density and uniform distribution throughout the scaffold. To achieve these conditions a custom-made perfusion chamber can be used.

Porous composite scaffolds consisting of polylactic acid (poly(95L/5DL)) and phosphate glass particles were obtained by solvent casting salt leaching method (porosity of 95.7% and a pore diameter of 80–210 µm, sample dimensions of 6 mm diameter and 12 mm length). For static seeding into scaffolds, MSCs in suspension were injected with a syringe into the scaffolds located in a Petri dish. For dynamic seeding scaffolds were put in the perfusion system and MG63 cell suspension was forced to oscillate through them with a fluid flow velocity of 1 and 10 mm/s at a frequency of 0.007 to 0.07 Hz during 465 cycles. Half of the scaffolds were examined under fluorescence stereomicroscope. The other half was frozen to break the cell membrane. Supernatants were collected and LDH activity was measured to quantify the cell number in the scaffolds.

Static cell seeding resulted in 32% efficiency, whereas dynamic seeding resulted in 36% and 55% efficiency with a velocity of 1 mm/s and 10 mm/s respectively.

These results show that the developed perfusion chamber induce higher cell seeding of the scaffolds. Further research in fluid flow velocity is undergoing to improve cell seeding and study cell differentiation under long-term culture.

(369) Stem/Progenitor Cell Mobilization for Tissue Regeneration

Lee S.J., Van Dyke M., Atala A., Yoo J.J.

Wake Forest Institute for Regenerative Medicine, Winston-Salem, North Carolina, United States of America

Introduction: It has been demonstrated that almost every tissue in the body contains some type of stem or progenitor cells. In this study we explored the possibility of utilizing host stem or progenitor cells for tissue regeneration *in vivo*. We investigated whether these cells could be mobilized into target specific sites for regenerative medicine applications.

Materials and Methods: Polyglycolide polymers were implanted subcutaneously in mice to recruit host cells. The scaffolds were retrieved at 1, 2, 3 and 4 weeks after implantation for analyses. Cells contained within the retrieved scaffolds were cultured and differentiated into osteogenic, endothelial, myogenic, and adipogenic cell lineages using the respective cell differentiation conditions.

Results and Discussion: The retrieved implants showed a progressive increase in neovascularization and host cell infiltration with time. Tissue infiltrates within the scaffolds showed the evidence of collagen deposition after 2 weeks of implantation and continued to increase over time. The culture of infiltrated cells showed the expression of stem cell markers, and these cells were able to differentiate into various cell lineages.

Conclusions: This study demonstrates that stem or progenitor cells can be mobilized and recruited into target specific sites. These findings suggest that host stem cells may be maneuvered to achieve tissue regeneration *in vivo* using a target specific scaffold system.

(370) Stimulating COMP Production in Cartilage Tissue Engineering

Bastiaansen-Jenniskens Y.M.^{1,2}, De Bart A.C.W.¹, Koevoet J.L.M.², Verhaar J.A.N.², Van Osch G.J.V.M.², De Groot J.¹

¹Business Unit BioSciences, TNO Quality of Life, Leiden

²Erasmus MC University Medical Center Rotterdam, The Netherlands

Cartilage Oligomeric Matrix Protein (COMP) is a protein present in the cartilage matrix. It binds to chondrocytes and collagen and is involved in interaction between chondrocytes and collagen and probably also in collagen fibrillogenesis. The present study was designed to investigate the effect of growth factors on COMP deposition and the influence of COMP on collagen deposition and mechanical properties.

Bovine chondrocytes in alginate beads were cultured with or without 25 ng/ml IGF1, TGFβ2 or FGF2. COMP deposition was determined with ELISA and Western blot. Human COMP (hCOMP) was also overexpressed in bovine chondrocytes using lentiviral transfection. hCOMP gene expression, COMP, collagen and proteoglycan deposition and mechanical properties were determined.

Addition of IGF1 and TGFβ2 resulted in more COMP than in the control condition without growth factors. Preliminary ELISA results support these findings. FGF2 resulted in less and partially degraded COMP. Lentiviral transfection with hCOMP resulted in elevated gene expression of hCOMP and increased COMP levels in the culture medium compared to untransfected cells. However, no difference was seen in the COMP deposition in the alginate bead. COMP overexpression also did not affect the deposition of collagen and proteoglycans or the mechanical properties.

It appears that COMP overexpression is not sufficient to increase extracellular COMP deposition in chondrocyte alginate culture. However, increased COMP deposition can be achieved by addition of growth factors, suggesting that modification of other factors is also needed to increase COMP deposition.

(371) Stimulation of Angiogenesis in an Enzymatically Cross-Linked Collagen Scaffold

Garcia Y.¹, Collighan R.², Griffin M.², Pandit A.¹

¹National Centre for Biomedical Engineering and Science, National University of Ireland, Galway, Ireland

²School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham, United Kingdom

One of the disadvantages of using collagen-based scaffolds as dermal substitutes is that the degradation rate does not equate to the impaired regeneration rate under pathological conditions. The introduction of chemical cross-linking agents to enhance enzymatic resistance has been shown to result in negative effects on cell viability. Microbial transglutaminase (mTGase) has been shown to provide *in vitro* collagen resistant bonds compatible with cell proliferation and differentiation [1, 2]. Therefore, it is hypothesised that mTGase cross-linked collagen scaffold may provide the same conditions *in vivo*, allowing infiltration of fibroblastic and endothelial cells as well as implant engraftment, thus constituting the ideal substrate to support dermal repair and regeneration. Native and cross-linked freeze-dried collagen type I scaffolds were fabricated and applied to full thickness 1 cm² wounds on the dorsum of Sprague Dawley rats (*n* = 6). Stereological methods were used to assess the cellular and tissue response. Specifically, wound closure, fibroblast infiltration, and acute inflammatory cellular response, epithelialisation rate and angiogenesis at three different time points (3, 7, 21 days) were assessed. The analysis of these parameters allowed us to conclude that enzymatically cross-linked collagen scaffolds stimulate wound bed angiogenesis and resist enzymatic degradation while fibroblast infiltration remains unaffected; properties that are considered ideal in a dermal substitute.

References

1. Chau, D. et al. (2005). The cellular response to transglutaminase-cross-linked collagen. *Biomaterials* 26:6518–6529.
2. Garcia, Y. et al. (In press). "Assessment of Cell Viability in a Three-Dimensional Enzymatically Cross-linked Collagen Scaffold." *J. Materials Science:Materials in Medicine*.

(372) Studying the Effect of Passaging Using a Thermoresponsive Surface Compared to a Tissue Culture Plastic Surface

Elowsson L.R.M., Crawford A., Hatton P.V.

University of Sheffield, Centre for Biomaterials & Tissue Engineering, Dept. Adult Dental Care, Claremont Crescent, Sheffield, S10 2TA, United Kingdom

Purpose: The aim was to investigate the effect of passaging on the chondrocytic phenotype using two types of surfaces, the regular tissue culture plastic (TCPS) surface and the commercially available culture plate RepCell® (TCS CellWorks Ltd, UK) with a thermoresponsive polymer-grafted surface, which allows culture

and detachment of cells without the use of animal-derived proteolytic enzymes.

Materials and Methods: Freshly isolated articular chondrocytes were seeded onto TCPS and RepCell® plates. The cells were cultured for 2-3 days until confluency was reached. Cells cultured on the TCPS plates were passaged using a trypsin/EDTA solution. Cells cultured on the RepCell® plates were passaged by thermal treatment (i.e. decrease of the temperature to below the lower critical solution temperature of the thermoresponsive polymer, approx. 32°C). Cell morphology was studied with light microscopy and live imaging during cell detachment using a fluorescence microscope after staining of the cells with CellTracker®. Actin fibre arrangement was studied by staining with X phalloidin. Cell viability was assessed by reduction of Alamar Blue™. Proliferation rate was studied by counting the cells.

Results and Conclusion: Data indicated that there was a difference in proliferation rate between chondrocytes cultured on the thermoresponsive-grafted surface of the RepCell® plate compared to chondrocytes cultured on TCPS, as well as a difference in the actin fibre arrangement. There may be a difference in chondrocytic phenotype, while scalloping of the cell membrane suggested that the enzymatic treatment had a negative effect on the cells. Further work is being undertaken to investigate gene expression by real-time PCR.

(373) Substrate Physicochemistry Controls Adhesion Force Balance During Matrix Reorganisation

Pompe T., Glorius S., Starruß J., Bobeth M., Pompe W., Werner C.

Leibniz Institute of Polymer Research Dresden, Max Bergmann Center of Biomaterials, Hohe Str. 6, 01069 Dresden, Germany
TU Dresden, Institut für Werkstoffwissenschaft, 01062 Dresden, Germany

Extracellular proteins are assembled into highly organised networks by adherent cells *in vivo* and *in vitro*. In tissue engineering applications using artificial scaffolds, the extracellular matrix represents the major joint at the interface of the biomaterial and the living tissue. However, in most cases the state of the extracellular matrix proteins is strongly affected by the biomaterials characteristics. These property changes are transmitted by the matrix to the attached cells and lead to downstream signalling events. In order to better understand the interplay of modified matrix properties on artificial substrates and cellular behaviour, we modulated protein-substrate interaction by gradually varying the physicochemical properties of maleic anhydride polymer substrates.

The reorganisation of the extracellular matrix protein fibronectin by endothelial cells into fibrillar patterns was quantitatively analysed by confocal laser scanning microscopy and scanning force microscopy. New features of the micrometer scale and nanometer scale pattern of fibronectin fibrils could be related to matrix-substrate interaction. The local as well as global force distribution along the cytoskeleton inside the cell together with the force-sensitive elements in the cell adhesion sites are suggested as the key elements in fibronectin pattern formation. The actin cytoskeleton structure and the magnitude of evolved forces are regulated by the fibronectin-substrate anchorage strength and by that determine the size and pattern of fibronectin fibrils. Adhesion force

measurements (centrifugation assay, traction force measurements) and nanometer scale simulations of fibronectin fibrillogenesis were performed in order to support the proposed mechanisms.

(374) Successful 3D Proliferation of Human Mesenchymal Stem Cells on Microcarriers

Schop D.¹, Janssen F.W.^{1,2}, Borgart E.¹, De Bruijn J.D.^{1,3}, Van Dijkhuizen-Radersma R.¹

¹R&D division, Progentix BV, Bilthoven, The Netherlands

²Department of Tissue Regeneration, Twente University, Enschede, The Netherlands

³Department of Materials, Queen Mary University of London, United Kingdom

Mesenchymal stem cells (MSC), e.g. from the bone marrow, have great potential as tissue regenerative source. However, they need to be isolated and expanded up to a million-fold for effective therapeutic use. In order to expand stem cells in a controlled, reproducible and cost-effective way, we are developing a bioreactor system based on microcarriers. In this study, the effect of cultivation conditions on cell proliferation and metabolism were investigated.

Human MSC, isolated from bone marrow of multiple donors, were seeded on Cytodex 1 microcarriers in spinner flasks (4.000 cells/cm², 20 cm² carrier/ml, 50 ml). Cells were expanded for 15 days under different cultivation conditions varying in feeding regimen (e.g. gradual addition of fresh medium and/or glucose solution). During cultivation, cell growth was followed using alamarBlue and light microscopy. Cell metabolism was monitored in terms of nutrient consumption (glucose, glutamine) and metabolite production (lactate and ammonia). After proliferation cells were characterized by adipogenesis, osteogenesis and chondrogenesis assays and FACS analysis (8 markers).

Continuous proliferation of the human MSCs on microcarriers was observed during the experiment. About 2 to 4 population doublings occurred, depending on the feeding regimen. Cell growth could be correlated to glucose consumption and lactate production. Interestingly, at low glucose concentrations lactate consumption was observed. High specific glucose consumption rates (qGlc \approx 8 pmol cell⁻¹ day⁻¹) and high yields of lactate from glucose (Ylac/glc \approx 2) were observed. Independent of the feeding regimen, glucose was preferred as energy source over glutamine. Multipotency and 'stemness' was proven for the proliferated cells.

(375) Surface Controlled Biomimetic Coating of Polycaprolactone Nanofiber Mesh Architectures

Araujo J.V., Martins A., Leonor I.B., Pinho E.D., Reis R.L., Neves N.M.

3B's Research Group—Biomaterials, Biodegradables and Biomimetics, Department of Polymer Engineering, University of Minho, Campus de Gualtar, Braga, Portugal
IBB—Institute for Biotechnology and Bioengineering, PT Government Associated Laboratory, Braga, Portugal

This work aims at developing novel electrospun nanofiber meshes coated with biomimetic calcium phosphate (BCP) mimicking the extracellular microenvironment found in the bone structure and not loosing the original morphology of the nanofibers. Poly(ϵ -

caprolactone) (PCL) was selected because of its current use in medical applications, biodegradability, biocompatibility and susceptibility to partial hydrolysis by alkaline treatments.

The deposition of a BCP layer, similar to the inorganic phase of bone, on PCL nanofiber meshes was achieved by a surface modification. This initial surface modification was followed by treatments with solutions containing calcium and phosphate ions. The process was finished by the immersion in simulated body fluid (SBF) with 1.5 \times the inorganic concentration of the human blood plasma ions. After an optimization stage, some conditions were selected for biological assays. The influence of the BCP on the viability, adhesion and proliferation of human osteoblast-like cells was assessed. It was shown that PCL nanofiber meshes coated with a BCP not only support but also enhance the proliferation of osteoblasts for longer culture periods.

The attractive properties of the coated structures produced demonstrate the potential of those materials as candidates for application in bone tissue engineering. This is to our best knowledge the first time that cell cultures were performed on nanofiber meshes coated with a bone-like BCP produced fully preserving the original mesh architecture.

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(376) Synthesis and Surface Activation of Synthetic Biodegradable Polymers as Support for Cell Produced ECM

Hilborn J., Atthoff B., Aulin C., Greis T., Aibibu D., Bolin J.

Materials Chemistry, Uppsala University, 751 21 Uppsala, Sweden

The aim of the EU-funded project 3G-SCAFF is to prepare a new type of scaffold and demonstrate in the animal model that it allows tissue repair and may be designed to promote regeneration.

Subsidiary objectives are to

1. Produce a polymer support part of the scaffold with adequate mechanical strength and mechanical properties matching the tissue to be repaired or regenerated.
2. Define bioreactor conditions for growth of cells on this support to deposit its own ECM
3. Production of a range of effective cell 'factories' with distinct functions by transfection of key effector/regulatory genes into appropriate fibroblast, epithelial and smooth muscle cell lines.
4. Assess functionality of the polymer supported, cell produced scaffolds in animal models.

To provide for the polymer support copolymerization of L-lactide with caprolactone was performed in kilo scale to produce polymers with 700,000 g/mol Mw (GPC) giving a melt viscosity suitable for processing to yarn. The multifilament yarn was knitted into porous compliant fabrics. To provide for cell attachment to these materials, they were subjected to surface hydrolysis followed by immobilization of collagen or fibrin. The so formed fabric scaffold allowed for (i) 3D cell seeding, (ii) support for ECM deposition by fibroblasts and (iii) reinforcement for compacted collagen and dynamically stimulated culture in bioreactors.

We believe that the combination of an engineered polymer support in combination with a designed ECM may be a suitable scaffold combining mechanical properties with biological cues to stimulate tissue regeneration.

(377) Synthetic and Natural 3D Scaffolds for MMSC in a Bone Defects Repair Experiments

Sviridova I.K., Sergeeva N.S., Reshetov I.V., Barinov S.M., Frank G.A., Tepljakov V.V., Kirsanova V.A., Akmedova S.A., Agzamov D.S., Filjushin M.M., Komlev V.S., Fadeeva I.V., Kozlov V.V., Bukharov A.V., Khesuani Yu. D.

Moscow Herzen Research Oncological Institute, Russian Federation
Institute for Physicochemical Problems of Ceramic Materials, Russian Academy of Sciences, Moscow, Russian Federation
Faculty of Basic Medicine, Moscow State University, Russian Federation

An effective way to deliver multipotent mesenchymal stromal cells (MMSC) into a bone lesion is a very relevant problem nowadays. The usage of nontoxic, biocompatible, bioresorbable 3D scaffolds for MMSC, that can provide optimal conditions for adhesion and expansion of immobilized cells and also promote a full integration of the implant into the surrounding tissue is actual.

Objectives: *In vitro* and *in vivo* study of porous granulated nanostructured bi-phase bioceramics and a natural coral to determine their usability as 3D matrixes; the usage of bioengineering construction (3D matrix with immobilized MMSC) to replace bone defects in experimental animals.

Materials and Methods: Investigating acute toxicity of the materials (24 hour incubation) and their matrix qualities (3–28 days of incubation) were performed during *in vitro* experiments on immortalized human fibroblasts by MTT-test. The biocompatibility was evaluated using *in vivo* experiments with female BDF1 mice which had the materials injected under their skin. Two experimental models, female Wistar rat shin osteotomy and segmental resection of sheep tibia and the autologous cultures of MMSC from bone marrow, were used to investigate the effectiveness of bioimplants. The dynamics of the bone defects repair are controlled by X-ray and a morphologic examination.

Results: *In vitro* experiments were showed that all samples were not cytotoxic and had marked matrix qualities, *in vivo* was found their biocompatibility. An osteoinductive potential of different extent was discovered during dynamic observation of rat shin and sheep tibia defects repair using bioceramic and coral implants without stem cells and in a bioengineering construction.

(378) Targeting Stem Cells Transfected with Magnetic Nanoparticles in a Flow System

Sura H.S., Dobson J., El Haj A.J.

Keele University, United Kingdom

Stem cell therapy has been proposed as a strategy for tissue repair and regeneration, and mesenchymal stem cells have been demon-

strated to migrate to sites of inflammation within the body. However, ensuring the cells remain at the site of therapy is not always possible. We describe an *in vitro* model for manipulating cells around a circulating system using magnetic nanoparticles with the application of external magnetic forces.

Stem cells were transfected with RGD coated particles (2–50 µg) (ø250 µm, Nanomod). Transfected cells with internalised particles were suspended in 2 ml of complete DMEM or porcine blood, injected into silicone tubing then incubated at 37°C and circulated in a specially designed flow system for 24 hours. Sintered zinc coated neodymium iron boron magnetic discs with a diameter of 7 mm and a thickness of 3mm were placed on the surface of the silicone tubing to trap circulating transfected cells. Three different flow rates were chosen to establish if there was a relationship between the flow rate and efficiency in trapping cells. SQuID analysis and confocal laser microscopy were used to quantify and characterise the trapped cells and LDH assays carried out to determine cell viability following treatment.

This study has shown that it is possible to manipulate stem cells labelled with magnetic nanoparticles remotely using a magnet. Trapping efficiency was affected by particle density, circulating media and fluid flow rate. Cell viability was unaffected by internalised particles. This feature may have future applications in tissue engineering and directed stem cell targeting for *in vivo* tissue repair.

(379) Temporomandibular Joint Reconstruction with the Aid of *In Vitro* Expanded Autologous Chondrocytes

Undt G., Fussenegger M., Marlovits S., Ewers R.

Hospital for Cranio-Maxillofacial and Oral Surgery, Medical University of Vienna, Austria
Institute for Reconstruction of Tissues and Organs (IGO®), Wels, Austria
Hospital for Traumatology, Medical University of Vienna, Austria

Summary of the Problem: Temporomandibular joint reconstruction in case of bony ankylosis still is a demanding procedure. We describe a new method of covering the articular fossa and the condylar head with constructs of a collagen-sponge, augmented with autologous, extracorporally augmented chondrocytes and fibrin glue.

Methods: From September to December 2003, five joints in three patients with temporomandibular joint ankylosis were reconstructed according to the new method. The patients collectively consisted of one man aged 46 and two women aged 36 and 66 years respectively. Three to five weeks prior to the surgical relief of ankylosis, autologous rib cartilage was harvested and digested in the laboratory. The chondrocytes were cultivated in autologous blood serum for three to five weeks. Following open relief of the bony ankylosis and contouring of the joint surfaces, the suspension of autologous chondrocytes was delivered to a collagen scaffold pre-shaped to cover the condylar head or the articular fossa at the skull base exactly.

Results: Histological examination of the biopsies taken from three joints of the two younger patients showed good differentiation of the tissues toward regular fibrocartilage four months after surgery. In all patients, the maximum inter-intercisal distance before surgery

(9, 10 and 15 mm) increased significantly following surgery and remained stable at the two years' control (25, 35 and 36 mm).

Discussion: Because of the good two-year clinical results and the histological findings at four months after primary surgery, we recommend the new method for reconstruction of the temporomandibular joint in case of ankylosis.

(380) Tethering of Designer Peptide Sequence on a Cross-Linked Collagen Scaffold

Damodaran G.¹, Collighan R.², Griffin M.², Pandit A.¹

¹National Centre for Biomedical Engineering, National University of Ireland, Galway, Ireland

²School of Life and Health Sciences, Aston University, Birmingham, United Kingdom

Collagen is widely used for delivering drug and therapeutic molecules. Laminin 5 is a basement membrane protein that regulates various cellular functions (1). The hypothesis of this research is that tethering modified laminin peptide 1 (PPFLMLLKGSTREAQQIVM) and peptide 2 (PPFLMLLKGSTRKKKKKG) to an enzymatically cross-linked collagen scaffold which will enhance cellular function. The designed peptides incorporated EAQQIVM, which are a substrate for transglutaminase and additional of lysine residues for cross-linking. The objective of this study is to investigate peptide 1 and 2 tethered to microbial transglutaminase (mTGase) cross-linked collagen scaffold for its cellular functions. Cell adhesion and spreading was characterised using May-Grunwald and Giemsa co-staining. The cytoskeleton of cells was visualised using FITC labelled phalloidin and collagen fibrillar assembly was studied by atomic force microscopy. The nature of cross-linking activity was studied using FTIR. Results indicate that both the modified peptide sequences support cell attachment and spreading without alteration in cytoskeleton. Alteration in fibrillar assembly that supports cell growth was seen with enzymatic cross-linking. Incorporation of laminin and modified peptide in mTGase cross-linked collagen scaffolds resulted in structural changes in the collagen scaffold, which indicates the extensive of cross-linking activity. From these results it is concluded that a tethered laminin peptide and modified peptide to the cross linked collagen scaffold shows potent cell adhesion and spreading.

Reference

1. Kim, J.M., et al. (2005) The PPFLMLLKGSTR motif in globular domain 3 of the human laminin-5 alpha3 chain is crucial for integrin alpha3beta1 binding and cell adhesion. *Exp Cell Res.* 2005 Mar 10; 304(1):317-27.

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(381) The Arteriovenous Loop Model in the Sheep; Up-scaling Towards Clinics

Beier J.P., Horch R.E., Hess A., Arkudas A., Polykandriotis E., Kalender W., Labahn D., Adamek E., Kneser U.

University Hospital Erlangen-Nuremberg, Department of Plastic and Handsurgery, Krankenhausstr. 12, 91054, Erlangen, Germany

The lack of vascularization still hampers the generation of bone tissue for reconstruction of larger bone defects. Hence new strategies for vascularized bone cultivation have been developed. The most promising could be the induction of axial vascularization by an arterio-venous loop (AV-loop). Encouraging results have until now only been obtained in small animal models. Here we present the very first application of the AV-loop model in clinically relevant dimensions, up-scaling it from rodents to sheep.

Hindlimb anatomy of German sheep was studied with special regard to an epifascial vascular axis, suitable for microsurgical creation of an AV-loop with sufficient length. An isolation chamber was developed for implantation in the sheep's groin embodying an AV-loop. Blood flow in the AV-loop was evaluated by different imaging techniques: duplex-scans, angio-CT and angio-MRI).

A constant vascular axis (one artery, two veins), was found at the sheep's thigh. The average length was 16 cm. AV-loops with diameters of 5 cm were successfully generated by anastomosing the artery to the vein. After placement into a Teflon chamber, we were able to visualize blood flow by duplex-scans, angio-CT and angio-MRT. The procedures were tolerated by all operated animals without any complications.

Up-scaling the AV-loop model from rodents to sheep might promise evaluation of axial vascularization within clinically relevant dimension. In the future, different suitable matrices will be applied for axial vascularization in the AV-loop sheep model. Once successfully employed in the sheep, clinical application of vascularized bone tissue engineering will be put into reach.

(382) The Attachment and Proliferation of Intervertebral Disc Cell on PLGA/SIS Hybrid Films

Ko Y.K., Kim S.H., Ha H.J., Lim J.Y., So J.W., Rhee J.M., Khang G., Kim M.S., Lee H.B.

BK-21 Polymer BIN Fusion Research Team, Chonbuk National University, Nanobiomaterial Lab., KRICT, Korea

We modified biocompatibility lacked PLGA film surface with small intestinal submucosa (SIS) in order to allow bioactivity. We evaluated the difference of adhesion and proliferation on intervertebral disc cells according to various contents of SIS in PLGA.

We separately isolated annulus fibrosus (AF) cells and nucleus pulposus (NP) cells from rabbit. 0.3 g PLGA was dissolved in 6 mL methylene chloride and mixed SIS powder according to content (0, 10, 20, 40 and 80 wt%). The solution was poured into glass dishes to form films. We carried out SEM for characterization of films. For the assessment of cell adhesion, AF and NP cells were seeded onto various films separately at 1×10^4 cells/cm². After 1, 2 and 3 days, we counted the number of adherent cells to confirm the initial cell attachment and proliferation. The morphology change of AF and NP cells was ascertained through SEM.

The PLGA film was smooth, however, PLGA film impregnated SIS has rough surfaces according to the content of SIS. We could confirm that SIS/PLGA film has higher AF and NP cell number than PLGA film, especially 10 and 20 wt% SIS. It might be explained that cytokines contained in SIS stimulate proliferation of IVD cells. SEM evaluation indicated that the cells in 10 and 20% SIS/PLGA film maintained their morphology. We concluded that SIS provides a proper substrate for disc cells.

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(383) The Cellular Response to a Polyurethane Surface Modified with Vascular Extracellular Matrix Proteins—Towards the Development of a Cell-Seeded Small Diameter Vascular Graft

Smith K.H., Jozwiak A.B., Bax D.V., Kielty C.M., Black R.A.

UK Centre for Tissue Engineering, Division of Clinical Engineering, Duncan Building, University of Liverpool, Liverpool, L69 3GA, United Kingdom

UK Centre for Tissue Engineering, School of Biological Sciences, Michael Smith Building, University of Manchester, Manchester, M13 9PT, United Kingdom

The aim of this work is to develop an artificial artery for use as a bypass graft. The hybrid artery consists of a porous tubular scaffold made from a polyurethane elastomer. The surface of the polymer is then modified with recombinant fragments of fibrillin-1 in order to encourage the growth of organised layers of vascular cells.

A recombinant fragment of fibrillin-1 was covalently bound to the surface of a polyurethane elastomer via a dextran intermediate. Human coronary artery smooth muscle cells were cultured on the modified surface. RGD-dependence of the cell attachment was investigated by incubating the cells with a soluble RGD-containing peptide prior to seeding. The contribution of integrins $\alpha v\beta 3$ and $\alpha 5\beta 1$ to the attachment of the cells was determined using blocking antibodies. Cell proliferation was quantified.

Cell attachment to the modified surface was found to be highly RGD-dependent and involved both integrins $\alpha v\beta 3$ and $\alpha 5\beta 1$, in agreement with earlier work where fibrillin-1 fragments were adsorbed onto tissue culture plastic (1). It follows that the surface modification procedure does not alter the conformation of the protein to the extent that its cell binding properties are diminished. Cells proliferated to a much greater extent on the protein-modified surface than on the untreated polyurethane.

We conclude that this surface modification protocol is an effective way to immobilise protein fragments to a polyurethane surface and that the presence of the fibrillin fragment increases adhesion and proliferation of arterial SMCs cultured on polymer surfaces.

Reference

1. Bax, DV, Bernard, SE, Lomas, A, Morgan, A, Humphries, J, Shuttleworth, CA, Humphries, MJ, Kielty, CM. 2003. J Biol Chem. 278. 34605–34616.

(384) The Comparative Analysis of Influence of TGF-beta1 and TGF-beta3 on Differentiation of MMSCs Isolated from Human Adipose Tissue into Cells of Cartilage Tissue in 2D and 3D Systems

Tepliyashin A.S.^{1,2}, Korjikova S.V.¹, Sharifullina S.Z.¹, Chupikova N.I.¹, Vasyunina N.Y.¹

¹“Institute of Stem Cell”, 107031, Russia, Moscow, Kuzneckiy Most str., 17/1

²“Beauty-Plaza” Medical Corporation, 107031, Russia, Moscow, Kuzneckiy Most str., 17/1

Introduction: In present time, development of new methodical approaches to use multipotent mesenchymal stromal cells (MMSC's) for regeneration of a cartilage tissue is extremely actual. The aim of our investigation was to select the optimal inductor for the directed differentiation of MMSC's from human adipose tissue (AT) into cells of a chondrogenic tissue in 2D- and 3D-systems.

Methods: MMSC's from AT were isolated by standard method and were stained by Ab's to Ag's: CD10, CD13, CD29, CD31, CD34, CD44, CD45, CD49a, b, d, f, CD54, CD73, CD90, CD105, CD117, CD133, CD166, HLA-ABC, HLA-DR,-DP,-DQ. MMSC's in pellet culture (2D) or in 3D scaffolds OPLA and Collagen I (BD Biosciences) after culturing in chondrogenic media for 4 weeks were analyzed by RT-PCR and immunohistochemical analysis to Aggrecan, CollagenII, and CollagenX. As the basic inductor of chondrogenesis was used TGF-beta1 or TGF-beta3 isoforms.

Results: Cells isolated from AT had phenotype of MMSC's. Induction of MMSC's differentiation *in vitro* these cells change their phenotype into cells of a chondrogenic tissue. Immunohistochemical analysis has revealed in both experimental groups presence of cells which positively stained with Ab's to CollagenII, Aggrecan and negative to CollagenX. The quantities of the cells involved into process of a differentiation in the media with TGF-beta3 were much more. Besides cells had more marked morphology of a cartilage tissue, formed lacunas and had bright expression of genes to the basic markers of chondrogenesis that supported by RT-PCR-analysis.

Conclusions: TGF-beta3 is more effective inductor of chondrogenesis for differentiation of MMSC's tissue in comparison with TGF-beta1.

(385) The Comparison Study on Collagen Scaffolds Seeded with Hair Follicle Epithelial Cells and Urothelial Cells

Drewa T.^{1,2}, Joachimiak R.¹, Kaznica A.¹, Wisniewska-Skopinska J.³, Sionkowska A.³

¹Department of Tissue Engineering, CM UMK, Karłowicza 24, 85-092 Bydgoszcz, Poland

²Chair and Department of Urology, CM UMK, Bydgoszcz, Poland

³Chair and Department of General Chemistry, UMK, Torun, Poland

Background: To develop a tissue-engineered bladder wall replacement with autologous cells and a biodegradable scaffold, both obtained from non-urinary tract components is an attractive idea. The aim of this study was to compare growth of rat hair follicle epithelial cells and rat urothelial cells on scaffold prepared from rat collagen.

Materials and Methods: Rat hair follicle epithelial cells and rat urothelial cells were both cultivated in DMEM (Sigma) supplemented with 10% of Fetal Bovine Serum (FBS) and EGF (10 ng/ml; Sigma). Cells were stained using anti-cytokeratine (CloneMMF) and anti-CD34 antibodies (Dako, Denmark). Collagen scaffold was prepared from tendons of Wistar rats' tails. 6-well plates were covered with collagen scaffold. 15×10^3 of hair follicle epithelial cells and urothelial cells were seeded on collagen surface and cultured for a week. Cells in the controls were seeded on polystyrene surface

of 6-well plates. After a week cell viability was assessed using MTT test (Sigma) and photo documentation was prepared.

Results: Hair follicle epithelial cells expressed epithelial markers and were slightly positive for CD34. Hair follicle epithelial cells proliferated very quickly. There were $292.5 \pm 33.3 \times 10^3$ and $167.4 \pm 24.9 \times 10^3$ cells growing on polystyrene and collagen, respectively. Urothelial cells expressed epithelial markers and were negative for CD34. These cells proliferated slowly. There were $40.0 \pm 4.2 \times 10^3$ and $4.5 \pm 1.8 \times 10^3$ cells growing on polystyrene and collagen, respectively.

Conclusions: Hair follicle epithelial cells can be potentially used in tissue-engineering, with the guarantee of the sufficient cell number for transplantation. Collagen scaffold slowed cell proliferation and probably influenced on cell differentiation process.

(386) The Development and Characterisation of Chondrocyte-Seeded Gradient Hydrogel Constructs for Cartilage Tissue Engineering

McLeod R., Pattappa G., Irianto J., Knight M.M., Lee D.A.

School of Engineering and Materials Science, Queen Mary University of London, Mile End Road, London, E1 4NS, United Kingdom

Articular cartilage is a heterogeneous tissue with alterations in chondrocyte morphology, matrix composition and mechanical properties with depth from the articular surface. Most chondrocyte-seeded constructs for cartilage tissue engineering, however, fail to take account of the inherent tissue heterogeneity. This study describes a novel method for the production of cell-seeded heterogeneous hydrogel constructs with defined graded properties.

A 2–4% (w/v) gradient agarose gel, seeded with 5×10^6 cells·mL⁻¹, was created using a commercially available gradient maker and methodologies modified from techniques used for electrophoresis. The constructs were cultured for up to 8 days and assessed for gross mechanical properties, molecular diffusion, cell viability, glycosaminoglycan (GAG) synthesis and cell deformation in response to the application of mechanical strain, using methods have been described in previous studies from the group.

The results indicated that the stiffness of the gel varied with position in the construct, with the greatest stiffness in the 4% region. The diffusion of a 500 kDa FITC-dextran exhibited an inverse linear relationship with concentration, such that the lowest rate of diffusion was apparent in the 4% region. Cell deformation, in response to gross compressive strain up to 20%, was greatest in the 2% region and was associated with alterations in local strain. No significant differences were found between the high and low concentration regions for either cell viability or GAG synthesis. In conclusion, this study demonstrates the successful production of hydrogel constructs with defined graded properties that may better replicate the heterogeneity within the native articular cartilage.

(387) The Development of a Muscle-Machine Interface for Use with Tissue Engineered Skeletal Muscle

Paxton J.Z., Donnelly K., Keatch R.P., Baar K.

Division of Molecular Physiology and Division of Mechanical Engineering and Mechatronics, University of Dundee, Dundee, United Kingdom

In vivo, the transition between muscle and bone is mediated by tendon, which eliminates the impedance mismatch between the compliant muscle and stiff bone. The aim of this study is to produce a cell-embedded scaffold to reproduce the muscle-machine interface *in vitro*. Scaffolds consisting of polyethylene glycol diacrylate (PEGDA) hydrogels structurally reinforced with hydroxyapatite (HA) and incorporated with the cell adhesion peptide RGD to improve cell attachment were produced for this purpose by UV crosslinking. The addition of HA alone or in combination with RGD did not affect the swelling properties of the hydrogel in either width or length. PEGDA-HA, PEGDA-RGD and PEGDA-HA-RGD hydrogels were seeded with primary tendon fibroblasts and cell counts were performed at 6, 24 and 48 hrs to quantify cell attachment and growth on the hydrogels. Highest cell attachment was observed on the PEGDA-RGD hydrogels, followed by PEGDA-HA-RGD gels. Unexpectedly, the PEGDA-HA hydrogels allowed cell adhesion and proliferation, suggesting that HA promotes cell adhesion, since PEGDA is non-adhesive to cells. The PEGDA-HA-RGD hydrogels were then cast in theta-shaped glass capillary tubes and loaded with primary tendon fibroblasts. After 4 days of incubation, sectioning and staining of the constructs showed DAPI and phalloidin staining primarily on the outside of the scaffolds, with isolated cells within the matrix. These preliminary studies suggest that the PEGDA-HA-RGD hydrogel may be a suitable scaffold for co-culture with an engineered muscle monolayer in the development of a muscle-machine interface to replicate the myotendinous junction, tendon and osteotendinous junction *in vitro*.

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(388) The Effect of Chitosan Powder on Human Fibroblasts and Keratinocytes *In Vitro*

Houghton T.C., Harris K.L., Sharpe J.R.

Blond McIndoe Research Foundation, Queen Victoria Hospital, East Grinstead, RH19 3DZ, United Kingdom

Chitosan has previously been shown to have potential as a low cost wound repair material, owing to its low cytotoxicity and ease of manufacture. It is also reported to have haemostatic and antimicrobial properties. We investigated the effect of different grades of powdered chitosan on human fibroblasts and keratinocytes *in vitro*. Three chitosans with varying degrees of deacetylation were investigated: practical grade (>85%), low molecular weight (75–85%) and high molecular weight (>80%).

Cell proliferation in the presence of powdered chitosan was studied using an anti-BrdU assay. The data showed a significant decrease in both keratinocyte and fibroblast proliferation when in contact with low and high molecular weight chitosan. Practical grade chitosan had no significant effect ($p > 0.05\%$ ANOVA) on keratinocyte proliferation and a small but significant effect ($p < 0.05\%$ ANOVA) on fibroblasts. For this reason practical grade chitosan was chosen for further study.

Glutaraldehyde was used to crosslink the chitosan powder for varying times and concentrations. A ninhydrin assay showed that crosslinking density was concentration dependant, and a reaction time of 5 minutes was sufficient to ensure maximum crosslinking. Chitosan crosslinked with glutaraldehyde above 0.2% was found to arrest proliferation of fibroblasts, as measured by anti BrdU assay.

Further studies will show whether this cross-linked chitosan has the same effect on keratinocytes.

Future work in this area will involve different cross-linking agents such as Genipin to develop a product capable of stimulating cell proliferation. This has important implications for the continued development of low cost wound repair materials.

(389) The Effect of P-15 on Human Dental Pulp Stem Cell Growth

Mohanram Y., Wood S., Kirkham J., Yang X.B.

Department of Oral Biology, University of Leeds, Leeds LS2 9LU, United Kingdom

Introduction: The development of autogenous stem cell and biomimetic biomaterial based therapy for bone augmentation is a major clinical need. The cell-binding domain of type 1 collagen, 15-residue peptide (P-15), has been shown to promote fibroblasts attachment and growth on anorganic bone mineral (ABM) and initiate the cascade of events leading to bone formation by human bone marrow stromal cells.

Aim: The aim of this study was to investigate the effect of P-15 absorbed ABM (ABM-P-15) scaffolds on human dental pulp stem cell (hDPSCs) growth and differentiation *in vitro*.

Methods: ABM and ABM-P-15 particles were provided by Cera-Pedics. hDPSCs were seeded onto ABM and ABM-P-15 scaffolds and cultured in basal media for up to three weeks. The cells attachment and proliferation on the scaffold were visualised by live/dead fluorescent markers and confocal and scanning electron microscopy. hDPSCs differentiation were confirmed by routine histology. In addition, the effect of P-15 on BMPs production by hDPSCs was analysed by C2C12 promyoblasts induction assay using co-culture method.

Results: P-15 promoted hDPSCs attachment and proliferation on ABM particles compare to ABM alone. hDPSCs cultured on ABM-P-15 scaffolds formed extensive cell bridges between the scaffold particles. Enhanced alkaline phosphatase expression of hDPSCs were observed on ABM-P-15 scaffolds compared to controls. P-15 stimulated hDPSCs BMPs production which promoted C2C12 promyoblasts differentiate along the osteogenic lineage.

Conclusion: P-15 absorbed ABM scaffolds appear to provide an ideal biomimetic microenvironment for human dental pulp stem cell growth and differentiation along the osteogenic lineage, offering new candidate material for bone regeneration.

(390) The Effects of a Supraphysiological Strain Magnitude, Combined with a Low Cycle Number on the Osteogenic Differentiation of Mesenchymal Stem Cells (MSCs)

Hampson K., Ashton B.A., El Haj A.

ISTM, Keele University, Thornburrow Drive, S-O-T, Staffordshire, ST4 7QB, United Kingdom

Although the peak functional strains in bones whose primary role is loading are 2000 to 3000 $\mu\epsilon$ experiments have found that under certain conditions bone cells *in vitro* require a much higher level of

strain to elicit an osteogenic response. These are known as 'supraphysiological' strains. The aim of this study was to determine if a supraphysiological strain magnitude, combined with a low cycle number, could enhance the biochemical osteogenic differentiation of MSCs.

MSCs at passage 4 were seeded onto cylindrical PLLA scaffolds (8 mm \times 10 mm) at 2×10^6 cells/scaffold and cultured for 7 days in the presence of 50 $\mu\text{g/ml}$ ascorbic acid-2-phosphate, 10 mM β -glycerolphosphate and 10 nM dexamethasone. A novel multi-sample rotating bioreactor was then used to apply a loading regime of 360 cycles/day for 5 days with a strain range of 1.7–4.2%.

The application of mechanical loading appeared to influence the behaviour of the MSCs but did not enhance their osteogenic differentiation; as shown by a decrease in the gene expression levels of bone sialoprotein, RUNX2 and Osterix and a significant decrease in alkaline phosphatase specific activities compared to unloaded controls. Although studies have shown that compressive loading regimes can enhance chondrogenesis there was no evidence to suggest that this loading regime induced the chondrogenic differentiation of these cells. Therefore the effect of this loading regime on the differentiation of MSCs needs to be investigated further.

(391) The Influence of Surface Biological Modification of Selected Implant Materials on the Viability of Bone Cells

Pešáková V.¹, Hulejová H.¹, Himmlová L.², Riedel T.³, Kubies D.³

Institute of Rheumatology, Na Slupi 4, Prague, 128 50, Czech Republic

The cellular response by which cells react to the presence of selected implant materials (zirconium ceramics, polished titanium, titanium with a rough treatment-sand blasted, synthetic hydroxyapatite coated on titanium base and carbon composite), covered with some blood components (plasma, serum), was studied under *in vitro* conditions.

The blood tissue is the site of the first connection between the foreign implant material and the live tissue of the recipient. The interactions between the material surface and blood are regulated by the physico-chemical parameters of the implant which, as has been evidenced in our previous work (Pešáková et al, 2007), play the key role. The influence of surface properties of implants covered with blood components, on response of cells growing in the implant immediate vicinity, namely on the adhesion, proliferation and synthetic activity of osteoblasts, was compared.

The implants were individually immersed into the human plasma and/or serum. On the surface of each sample has arisen thin layer of protein network. Subsequently all samples were seeded with osteoblasts (NHOst Cambrex, USA, 3rd passage) and were cultivated under the standard conditions. We monitored a direct influence of the covered implant materials on the cell viability using the MTT test according to Laughton (1984). The presence of some inflammatory mediators was evaluated in the cultivation medium using ELISA methods.

The plasma coating increased the osteoblast proliferation primarily on titanium material hydroxyapatite coated. In this case the proliferation was higher and the expression of inflammatory mediators decreased.

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(392) The Relationship Between Epithelial Outgrowth and the Size and Conformation of *Ex Vivo* Skin Explants

Bainbridge N.J., Harris K.L., Sainsbury D., Tay S., Knox P., Sharpe J.R.

Blond McIndoe Research Foundation, Queen Victoria Hospital, East Grinstead, RH19 3DZ, United Kingdom

Skin explants in culture provide an invaluable *ex-vivo* model and give an insight into the behaviour of skin during wound healing. Information gained using explant models can be used to optimise skin grafting, develop biomaterials, wound dressings and tissue engineered skin.

Split thickness skin (STS) (200–250 μm) was obtained using a dermatome from human skin discarded following routine surgery. Round explants of varying sizes, ring-shaped explants (created using biopsy punches and trephines), and oblong explants of varying lengths and widths were created from the STS. Explants were fixed, stained using Coomassie Brilliant Blue, photographed and analysed for cell sheet outgrowth by image analysis.

Outgrowth of keratinocyte cell sheets from explants was found to be inversely proportional to explant size. An explant with an area of 0.015 cm^2 had an area of keratinocyte outgrowth that was 4 \times greater than that of an explant with an area of 0.230 cm^2 . Ring-shaped explants showed more keratinocyte outgrowth on external surfaces than on the interior surfaces of the holes. Oblong shaped explants showed outgrowth from the ends of the explant but not from the central region.

These preliminary results suggest that cell sheets grow preferentially and more rapidly from areas of explant which have a high peripheral area relative to their volume, which has great relevance to current skin grafting techniques. The sizes, shape and peripheral area to volume ratio of meshed skin grafts may have a large effect on the rate of cell sheet outgrowth and therefore wound closure in patients receiving skin grafts.

(393) The Relationship Between Tendon Composition, Structure, and Mechanical Characteristics

Toorani S., Shelton J.C., Screen H.R.C.

QMUL, School of Engineering and Material Science, Mile End Road, London, E1 4NS, United Kingdom

Tendons transfer muscle forces to the skeleton, providing specialised functional characteristics (1, 2). Tendon is a prime candidate for tissue engineered replacement due to its relatively avascular nature. Previous attempts to create a compatible constructs have had limited success as each tendon has a unique mechanical role. This study hypothesises that the functional requirements of tendons dictate an optimised composition and structure.

Fascicles were dissected from porcine flexor and extensor tendons. Flexor ($n = 22$) and extensor ($n = 29$) fascicles were subjected to quasi-static loading at strain rates of $10\% \cdot \text{min}^{-1}$ until failure. In a separate study fascicles ($n = 30$) were lyophilised and digested prior to a DMB assay, to determine water and GAG content (3). Also flexor and extensor fascicles ($n = 2$) were fixed in 4% glutaraldehyde, embedded, sectioned and stained to image crimp.

The flexor fascicles demonstrated a higher UTS ($48.12 \pm 3.03 \text{ MPa}$) and modulus ($265.5 \pm 48.20 \text{ MPa}$) than extensors

(UTS = $35.56 \pm 2.59 \text{ MPa}$, $E = 230.07 \pm 21.21 \text{ MPa}$). The water content of flexors (68.2%) was lower than extensors (72.2%), and flexors contained less GAG (0.46%) than extensors (0.76%). Flexor fascicles exhibited more tightly packed fibres with a sharper crimp angle (153°) compared to (166°) extensors.

This study has identified structural and biochemical differences between flexor and extensor tendons, showing subsequent differences in their mechanical behaviour. Further work is required to establish how differences are controlled by the cells.

References

1. Józsa L, Kannus P (1997) Human Tendons. Pub: Human Kinetics.
2. Batson EL et al. (2003) Equine Vet. J. 35(314–8).
3. Farndale RW et al. (1982) Conn. Tiss. Res. 9(247–8).

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(394) The Response of Cells from Mesenchymal Origins to Osteogenic Stimuli After Sub-culture *In Vitro*

Hampson K., El Haj A., Ashton B.A.

ISTM, Keele University, Thornburrow Drive, S-O-T, Staffordshire, ST4 7QB, United Kingdom

Bone tissue engineering strategies often require cells to undergo extensive expansion *in vitro* due to the need for larger implants to replace damaged bone tissue. Although the main source of osteoprogenitors is in the bone marrow some *in vitro* studies have investigated the use of alternative mesengenic cells including osteoblast-like cells and chondrocytes. This study compares the osteogenic potential of the mesenchymal cells, stem cells (MSCs), bone cells and chondrocytes after expansion *in vitro*.

Passage 4 MSCs, bone cells or chondrocytes were plated at 5000 cells/ cm^2 and cultured for 7, 14, 21 and 28 days in Dulbecco's modified Eagle's medium with or without osteogenic supplements; 50 $\mu\text{g}/\text{ml}$ ascorbic acid-2-phosphate, 10 mM β -glycerophosphate and 10 nM dexamethasone.

The MSCs responded to long term culture in the presence of osteogenic supplements with an increase in alkaline phosphatase specific activity, calcium accumulation and the expression levels of osteogenic markers. Although the bone cells also showed enhanced osteogenesis in response to the osteogenic supplements their response was greatly reduced compared to the MSCs. The chondrocytes showed no conclusive evidence of osteogenesis after long term culture in the presence of osteogenic supplements as demonstrated by a limited up-regulation of osteogenic genes.

This study demonstrates that the most promising source of osteoprogenitors after sub-culture *in vitro* are MSCs.

(395) The Use of Osteoblast/Osteoclast Co-cultures on PLLA, Silk and Dentine Scaffolds

Jones G.¹, Marshall M.³, El Haj A.J.¹, Motta A.², Cartmell S.¹

¹ISTM Keele University, Thornburrow Drive, Stoke on Trent ST4 7QB, United Kingdom

²DIMTI University of Trento, via Mesiano 77, 38050 Mesiano (TN), Italy

³Charles Salt Centre, RJA Orthopaedic Hospital, Oswestry, Shropshire, SY10 7AG, United Kingdom

Tissue engineering of bone has traditionally been approached using a single cell (osteoblast or progenitor) culture in a 3D scaffold model. We are aiming towards a co-culture protocol that can more accurately reflect the remodeling *in vivo* environment. With the addition of a biodegradable scaffold, the potential relationship in turnover between new matrix (ECM) formation, matrix degradation and scaffold degradation can be investigated *in vitro* using our model.

Murine monocytes were enzymatically differentiated toward multinucleated osteoclasts (TRAP +ve). The osteoclasts were cultured for 7 and 14 days on PLLA, dentine and silk fibroin films. Murine osteoblasts were also cultured on the same scaffold types over a 7 and 14 day period.

Scaffold degradation has been evaluated by micro CT, SEM, AFM and DSC. Cell activity has been determined using TRAP, alkaline phosphatase and hydroxyproline assays.

Results from MicroCT show that degradation in dentine slices occurred over time with a 2% decrease in volume in the presence of osteoclasts. Increased roughness of the surface of the dentine by 400% and 200% after 14 days in presence and absence of osteoclasts respectively by AFM.

A co-culture on the three scaffolds is now being optimized by varying the ratio of osteoclast/osteoblast density on each scaffold with further evaluation of ECM production versus scaffold degradation. In this way, we can begin to assess the relationship between cell/cell interaction and cell/scaffold interaction for bone tissue engineering.

(396) The Use of Statins to Enhance Bone Mineralisation and Formation in 2D and 3D Tissue Engineered Constructs

Griffiths S., Cartmell S.

Keele University, Institute of Science and Technology in Medicine, Guy Hilton Research Centre, Thornburrow Drive, Stoke-on-Trent, ST4 7QB, United Kingdom

The culture of human bone cells for tissue engineering has great potential as a therapy for patients suffering from bone loss due to disease or trauma. This study has looked at using simvastatin, for the novel use of enhancing human bone cell tissue engineered constructs *in vitro*.

Statins are generally prescribed to lower cholesterol, but can also enhance bone differentiation, maturation and matrix formation, with increased BMP2 mRNA locally. Our study used simvastatin as a media supplement for *in-vitro* 2D and 3D primary human osteoblasts and osteosarcoma cell line seeded constructs, utilising gene expression analysis via real-time RT-PCR, picogreen DNA assays, with MicroCT and calcium assay mineralization data. Results of 2D cultures show low statin concentrations (0.001 microM) have a significantly increased proliferative effect in 3 and 7 day cultures, whilst higher optimised simvastatin concentrations (5 microM) significantly reduce osteoblast proliferation, with increasing osteoblast differentiation and maturation at 7 days. 3D scaffold cultures showed increasing volumes of mineralised matrix production per cell in primary human cell cultures, using 5 microM simvastatin as a media supplement. The osteosarcoma human cell line MG63 also showed significantly decreased proliferation with 5 microM simvastatin, however at 3 days, compared to primary cells 7, which then regained proliferative capacity in 7 day culture. These results suggest that various statin concentrations could be used with non-diseased bone tissue to enhance proliferation, dif-

ferentiation and mineralisation of bone cells, when introduced at various culture periods to enhance tissue engineered constructs, but could also be utilised in bone cancer therapies.

(397) Thermoreversible Poly(N-isopropylacrylamide)-g-Methylcellulose Hydrogel for Cartilage Tissue Engineering

Lima H.C., Tuzlakoglu K., Mano J.F., Reis R.L.

3B's Research Group—Biomaterials, Biodegradables, and Biomimetics, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

IBB—Institute for Biotechnology and Bioengineering, PT Government Associated Laboratory, Braga, Portugal

Damaged or degenerated articular cartilage has a limited ability of self-regeneration response, where repair is still a challenging clinical problem. Tissue engineering (TE) can offer alternative solutions to current cartilage-repair techniques. A variety of materials have been suggested for cartilage repair, and injectable hydrogels are among the most promising alternatives.

The aim of this work is to explore the ability of Poly(N-isopropylacrylamide)-g-Methylcellulose (PNIPAAm-g-MC) thermoreversible hydrogel as a scaffold material for cells encapsulation to regenerate cartilage through a TE approach by using ATDC5 chondrogenic cells.

The PNIPAAm-g-MC hydrogel was successfully synthesised using ammonium-persulfate and N,N,N',N'-tetramethylethylenediamine as initiator. FTIR results confirmed the formation of the copolymer. The temperature responsiveness of the copolymer was investigated: it became gel at temperatures above its lower critical solution temperature (LCST), whereas liquefying at temperatures below its LCST (around 31°C, as measured by DSC).

ATDC5 cells, a murine chondrogenic cell line, were used as *in vitro* model for this study. They were encapsulated at high cell density within the hydrogel and cultured for up to 28 days with basal medium. PNIPAAm-g-MC hydrogel did not affect the cell viability and proliferation, as indicated by both MTS and DNA assays. The results also revealed an increase in the synthesis of glycosaminoglycans (GAG) at 28 days by dimethylmethylene blue (DMB) quantification assay.

These preliminary results suggest the potential use of PNIPAAm-g-MC thermoreversible hydrogel as three-dimensional scaffold for cartilage TE using minimal-invasive strategies.

(398) Three Dimensional Polymer Scaffolds Fabricated by Surface Selective Laser Sintering (SSLS)

Upton C.E., Howdle S.M., Shakesheff K.M., Popov V.K.

Schools of Chemistry and Pharmacy, University Park, University of Nottingham, NG7 2RD, United Kingdom

SSLS is a relatively new method for fabricating 3D composite scaffolds that are both bioactive and biodegradable (1). This method uses a laser to fuse together polymer particles into intricate scaffolds. Research has shown that SSLS scaffolds are suitable for bone regeneration and initial studies show that C2C12 cells can be induced to form viable osteogenic cells on SSLS poly(D,L-lactic acid) (PDLLA) scaffolds. However, for optimal bone repair the scaffold ideally needs to be fabricated with high precision and accuracy in order to fit anatomically into a defect. Using SSLS it is

possible to produce 'made to measure' scaffolds but the resolution of the final scaffold is currently restricted by the size and morphology of the sintered polymer particles.

In this study we utilise a process known as particles from gas saturated solutions (PGSS) to produce polymer microparticles using supercritical carbon dioxide (scCO₂) (2). Thus, in order to further understand the mechanism of particle formation from scCO₂, we have utilised high-pressure rheometry to measure the viscosity of polymers in scCO₂ *in situ*. The results reported show how varying temperature, pressure, molecular weight and polymer soaking time in scCO₂ effect the viscosity of the polymer. Measuring polymer viscosity in scCO₂ is shown to be a useful tool to predict the morphology of products obtained by PGSS and has aided in our development of well-defined particles for SSLs.

References

1. E.N. Antonov, V.N. Bagratashvili, M.J. Whitaker, et al (2005) Adv. Mater. 17(3) 327–330.
2. S.M. Howdle, M.S. Watson, M.J. Whitaker, et al (2001) Chem. Commun. 109–110.

(399) Three-Dimensional Modelling of the Transversalis Fascia in Hernia

James R.¹, Trwoga P.², Brown R.A.³, A. Afoke A.²

¹University College London, Department of Electronic and Electrical Engineering, Torrington Place, London WC1E 7JE, United Kingdom

²University of Westminster, School of Informatics, Cavendish Campus, London W1W 6UW, United Kingdom

³University College London, Tissue Repair and Engineering Centre, Institute of Orthopaedics & Musculoskeletal Science, Stanmore Campus, London HA7 4LP, United Kingdom

The transversalis fascia is implicated in the onset of hernia and a recent study of its material properties has shown that it is anisotropic in nature (Kureshi et al., private communication). Due to the nature of the problem, it is difficult to quantify the stresses and strains present in the fascia during the continuous protrusion of the fascia in the advanced stage of the hernia. However, this presents an appropriate subject for computer modelling, the results of which are relevant in the development of tissue engineered fascia (Kureshi et al.¹).

Our approach is to model the protrusion of the fascia using three dimensional finite element methods based on the anisotropic theory of elasticity. A variational approach is used to find the displacement that minimises the total potential energy and the resulting stress distribution is then visualised over the surface of the protrusion.

Reference

1. A. Kureshi, Z. Hyder, A. Afoke, R.A. Brown; Use of plastic compressed collagen sheet scaffolds to engineer a model of fascia tissue. Submitted to TERMIS-EU2007.

(400) Thrombospondin-1 Mediates the Biomechanical Response of Fibroblasts Within Contracted Collagen Matrices

Chen Y.L., Abraham D., Leask A., Xu S.W., Denton C., Black C., Eastwood M.

School of Biosciences, University of Westminster, 115 New Cavendish Street, London, W1W 6UW, United Kingdom
Centre for Rheumatology, University College London, Royal Free Campus, Rowland Hill St., London, NW3 2PF, United Kingdom

Fibroblasts are able to respond to their environment and to rapidly changing biomechanical loads such as during wound healing, tissue repair and fibrosis, by initiating transcriptional programs that determine biosynthetic activity and differentiation status. We have utilised 3-D fibroblast populated collagen lattices (FPLC) to assess changes in gene expression profiles and functional activities during contraction and under a defined biomechanical regimes that mimic fibrosis *in vitro*. Using Affymetrix U133 gene chips, we found significant differences between control fibroblast in non-contracted gels compared to fibroblasts in contracted gels and those exposed to precise biomechanical loading regimes. Approximately 400 of the 12,000 genes analysed were differentially expressed in the fibroblast samples. Gene cohorts included proteases, structural proteins (myosin, smoothelin, thrombospondin-1; TSP-1), growth factors (CTGF) and transcription factors (c-fos). TSP1 a matricellular protein is expressed in many fibrogenic diseases, and is a known activator of latent TGF- β . The presence of a TSP-1 blocking peptide significantly reduced the biomechanical force generated by control and scleroderma fibroblasts to a level similar to that found in the presence of the TGF β receptor type I, ALK-5 inhibitor. Our data suggest that biomechanical loading induces the expression of TSP-1 which plays a central role in controlling the level of active TGF- β and subsequent fibroblast response.

(401) Thyrocyte Sheets Fabricated by Cell Sheet Engineering and Their Transplantation to the Hypothyroidism Model

Arauchi A., Shimizu T., Yamato M., Obara T., Okano T.

Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan

For some patients received total thyroidectomy, oral administration is sufficient to restore thyroid hormone levels. We propose a tissue engineering approach to treat the consequences of total thyroidectomy. For this purpose, temperature-responsive culture dishes were used to prepare multi-layered thyroid cell sheets to be transplanted in a model of total thyroidectomy. Thyroid glands were obtained from 4-week-old Lewis rats. The cell suspension was cultured on temperature-responsive culture dishes. When the cells reached confluence, the dishes were moved to 20°C, to allow the detachment of thyroid cell layer. The 8-week-old Lewis rats were exposed to total thyroidectomy as hypothyroidism models and received triple-layer thyroid cell sheets transplantation one week after thyroidectomy.

One week after total thyroidectomy, serum levels of fT3 and fT4 appeared significantly decreased. But the transplantation of triple-layer thyroid cell sheets was able to restore thyroid function, as shown by the enhancement in serum levels of thyroid hormones one more week after the cell sheets transplantation. Moreover, histological analyses were performed on the resected grafts by Hematoxylin/Eosin staining showed that the samples displayed typical thyroid follicle organization and anti-TTF-1 antibody staining showed the presence of follicle epithelial cells. The presence of numerous functional microvessels containing red blood cells was also detected. Thus, our results indicate that thyrocyte cell sheets transplanted in a model of total thyroidectomy histologically

resemble thyroid gland organization and are able to restore thyroid function *in vivo* and suggest that thyroid cell sheets could be suitable for the therapy of post-operative hypothyroidism.

(402) Tissue Engineered Arthroscopic Cartilage Repair in Horses

Masri M., Ibarra C., Lombardero G., Velasquillo C.

Universidad Nacional Autónoma de México Facultad de Medicina Veterinaria y Zootecnia Instituto Nacional de Rehabilitación, Mexico

Objective: The purpose of this study was to repair experimental articular cartilage lesions in horses by arthroscopic implantation of autologous chondrocytes expanded *in vitro* and seeded onto polyglycolic/poly(lactic acid) (pgla) and collagen scaffolds.

Methods: Twenty horses were used, divided into three groups. Group 1: 6 horses implanted with a chondrocytes seeded onto pgla discs. Group 2: 6 horses implanted with a pgla disc encapsulated with a chondrocyte monolayer. Group 3: 6 horses implanted with a collagen disc encapsulated with a chondrocyte monolayer. Controls (cartilage lesions with a drill hole in the subchondral bone) and osteochondral defects (donor sites) were also evaluated. Two horses were implanted with a construct of autologous chondrocytes transfected with Ad-GFP vector to verify presence of autologous chondrocytes in the repair tissue. The cell-polymer constructs were implanted arthroscopically and fixed to the subchondral bone with biodegradable suture anchors. After 8 weeks (6 horses) and 6 months (12 horses) repair tissue was evaluated by arthroscopy using the ICRS and Oswestry's criteria. Two punch biopsy specimens per horse were evaluated histologically using H&E, safranin-O and trichomic stains to assess morphology, and determine the presence of proteoglycans, collagen, and evaluate integration to the surrounding cartilage.

Results: All specimens retrieved showed the presence of repair tissue within the experimental lesions. Experimental groups showed significantly better tissue quality under all parameters evaluated than controls. Encapsulated PGLA constructs showed the best tissue characteristics between experimental groups.

Conclusions: experimental cartilage lesions in horses were repaired with cartilage-like tissue by arthroscopic implantation of autologous chondrocytes seeded onto biodegradable scaffolds.

(403) Tissue Engineered Bone Marrow Mesenchymal Stem Cells in the Construct Polycaprolactone-Pluronic-F127

Chung H.Y., Lin T.M., Chang S.C.N., Jeng L.B.

China Medical University Hospital, Taichung, Taiwan

Bone marrow comprising heterogeneous cell populations contains certain progenitors with the ability to differentiate into multiple mesenchymal cell lineages. Bone marrow mesenchymal cells (MSC) will grow better if the construct are made to facilitate for their growth. Polycaprolactone (PCL) is a biodegradable polymer which is non-toxic, biodegradable, broad miscibility or mechanical compatibility with many polymers and good adhesion to a broad

spectrum of substrate. PCL has been used as a drug release biomaterial. Pluronic F127 (Pluronic) is composed of PEO-PPO-PEO (poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide)) which is solvable, biocompatible and nontoxic. To investigate the growth of MSCs in porous construct, twelve different compositions of PCL-Pluronic were tested.

MSCs of miniature swine were aspirated from iliac crest and culture on the PCL-Pluronic. The PCL-Pluronic copolymers were composed in different concentration as 9:1, 8:2, 7:3 and 6:4 so that they will be able to sustain shaping. Three temperatures during proceeding the co-polymer were tested as 4°C–20°C and room temperature. We will perform SEM, confocal study and DNA analysis. The results have shown that PCL-Pluronic is a good biocompatible biomaterial. The MSC number is high in the –20°C 6:4 composition after day 6 *in vitro*. The cellular growth accelerated because there were uniform pores in the optimal composition compared with those made in 4°C and room air. With the aid of hydrophilic Pluronic, the hydrophobic PCL may provide a better surface for MSC attachment. Further *in vivo* study in minipigs will be performed.

(404) Tissue Engineered Canine Cranial Bone Regeneration with the Concept of Guided Bone Regeneration

Umeda H., Kanemaru S., Yamashita M., Ohno T., Suehiro A., Tamura Y., Hirano S., Nakamura T., Omori K., Ito J.

Department of Otolaryngology and HNS, Graduate School of Medicine, Kyoto University, Japan

Objective: To estimate the effectiveness of calcium alginate membrane (CAM) in keeping bone regenerative space. We previously reported skull regeneration using the bone substitute material (BSM) which consisted of collagen coated beta-tricalcium phosphate, autologous bone fragments and bone marrow derived stromal cells (BSCs). However, excessive fibrous tissue intrusion or defluxion of BSM sometimes interrupted the bone regeneration. To prevent such disadvantages, we examined CAM, which was reported useful for guided bone regeneration (GBR).

Study design: Preliminary: an animal experiment.

Materials and Methods: Adult beagle dogs were used. The bone defect (2 cm×2 cm) was created as the same clinical procedure. Four experimental models were designed with or without BSM or CAM. In group I, the original free bone flap was only replaced at the defect. In group II, after replacing the bone flap, the defect was covered with the CAM. In group III, BSM were used as the filler of the gap. In group IV, BSM and CAM were applied. Histologic examinations three months after the operation were done.

Results: In groups I and II, bone regeneration wasn't observed. Fibrous tissue intrusion was found in group I. Neogenesis of bone was observed more widely in group III than in group IV. However, less fibrous tissue intrusion was observed in group IV than in group III.

Conclusion: CAM has the possibility to prevent excessive fibrous tissue intrusion and/or defluxion of a scaffold. However the delay of the neogenesis was observed.

(405) Tissue Engineered Tubularized Urethra for Surgical Reconstruction: A Pre-Clinical Study

Orabi H.O., Aboushwareb T., Yoo J.J., Atala A.

Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, United States of America

Introduction: Various urethral conditions often require extensive surgery. The treatment options for patients requiring repair of a large segment of tubularized urethra are limited by the availability of autologous tissues. In this study we explored the feasibility of engineering clinically relevant long urethras for surgical reconstruction in a canine pre-clinical model.

Materials and Methods: Autologous bladder epithelial and smooth muscle cells were grown and seeded onto collagen based tubular matrices (6 cm in length). The urethral segment of approximately 6 cm in length was removed in 18 male dogs and replaced with the cell seeded collagen scaffolds in 12 animals, and without cells in 6 animals. The animals were sacrificed at 1, 3, 6 and 9 months for analyses.

Results and Discussion: The CT scan showed wide caliber urethras in the animals implanted with the cell seeded matrices. The urethral segments replaced without cells had tissue breakdown and scaffold collapse. The urethral implants seeded with cells showed normally appearing tissue without evidence of fibrosis grossly. Histologically, formation of an organized urethral tissue structure was evident in the cell seeded constructs.

Conclusions: These findings show that long segments of cell seeded tubularized scaffolds can be used to repair large urethral defects. This study demonstrates that tissue engineered tubularized long urethras may be used for surgical reconstruction in patients requiring urethral tissue repair.

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(406) Tissue Engineering of a Vascularised Myocardial Patch

Morritt A.N.^{1,2}, Bortolotto S.K.¹, Dilley R.J.¹, Han X.L.¹, Kompa A.R.³, McCombe D.^{1,2}, Wright C.E.⁴, Itescu S.³, Angus J.A.⁴, Morrison W.A.^{1,2}

¹The Bernard O'Brien Institute of Microsurgery, Melbourne, Australia

²The Departments of Surgery and ³Medicine, St Vincent's Hospital, Melbourne, Australia

⁴Cardiovascular Therapeutics Unit, Department of Pharmacology, University of Melbourne, Australia

Introduction: An engineered myocardial patch could potentially be used in the treatment of acquired and congenital cardiac defects. Hypoxic limitations mean that only thin layers of functional tissue have been generated *in vitro*. Using an *in vivo* approach, we have created vascularised, beating cardiac muscle as thick as the adult rat right ventricle wall [1].

Methods: Neonatal rat cardiomyocytes, Matrigel and an arteriovenous blood vessel loop were incubated in a chamber in the groin

of an adult rat. Chamber tissue was harvested up to 10 weeks later, and underwent histological and functional analysis.

Results: Constructs harvested at 4–10 weeks contracted spontaneously and contained a profusely vascularised interconnected network of cardiomyocytes. Extracellular matrix deposition was similar to intact adult rat ventricle, and lymphangiogenesis was evident throughout the tissue construct. Cardiac tissue growth was supported by hypertrophy and proliferation. Organ bath experiments showed a typical cardiac muscle length-tension relationship, positive chronotropy to norepinephrine, positive inotropy to calcium and ability to be paced electrically.

Conclusion: Vascularised, spontaneously beating cardiac tissue was generated by incubating neonatal rat cardiomyocytes in a vascularised tissue engineering chamber.

Reference

1. Morritt, A.N., et al. (2007). Cardiac tissue engineering in an *in vivo* vascularized chamber. *Circulation* 115(3): p. 353–60.

(407) Tissue Engineering of Skin: Are Compressed Collagen Gels the Key to Success?

Fredriksson C.^{1,2}, Huss F.², Hadjipanayi E.³, Brown R.A.³

¹KaroCell Tissue Engineering AB, Stockholm, Sweden

²Institution of Biomedicine and Surgery, Department of Experimental plastic surgery, Faculty of Health Science, University of Linköping, Sweden

³University College London, Tissue Regeneration & Engineering Centre, Institute of orthopaedics Campus, London, HA7 4LP, United Kingdom

Tissue engineered skin substitutes often rely on the ability of cultured cells to adhere to the scaffold; to interact with the host tissue, and ultimately to regenerate tissue *in situ*. The success rate can be limited and in view of that there is a constant drive to identify novel manipulations to improve the degree of integration between a tissue engineered construct and the host. Previous reports on plastic compression (PC) of hyperhydrated collagen gel has been promising, as they rapidly produce a dense, mechanically strong, scaffold with high cell viability [1]. With the prospect of further developing this into a skin substitute, we studied the possibility of human keratinocytes forming stratified layers on compressed collagen gels. Human primary fibroblasts were seeded in the collagen gels and after compression the construct surface was seeded with human primary keratinocytes. The constructs were cultured, submerged in media for two days, raised to gas/liquid interface, and cultured for an additional 6–10 days. Constructs rapidly took on the histological appearance of a skin model. Immunolocalisation for type IV collagen and laminin-5 suggests that there was little epidermal-dermal junction (EDJ) basement membrane formation at this early stage. It is concluded that PC collagen with a keratinocyte surface layer represents an effective, new and rapid route to generate skin-like constructs though introducing a 3D ridge structured collagen-epidermis interface may improve biomimesis.

Reference

1. R.A. Brown et al. 2005. Ultra Rapid Engineering of Biomimetic Materials and Tissues: Fabrication of Nano- and Microstructures by Plastic Compression. *Adv Fund Mater.* 15. 1762–1770.

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(408) Tissue Engineering of Small Calibre Vascular Grafts

Tschoeke B., Sriharwoko M., Ellä V., Koch S., Glitz A., Schmitz-Rode T., Gries T., Kellomäki M., Jockenhoevel S.

Applied Medical Engineering, Helmholtz Institute Aachen, Pauwelsstr. 20, 52074, Aachen, Germany

Atherosclerosis is the most frequent cause of mortality in Western societies. In addition to autologous vascular grafts and synthetic vascular grafts, cardiovascular tissue engineering promises new sources for vessel transplants. The necessity of a short production time led to the concept of the Vascular Composite Graft with the advantages of viable tissue and with optimal mechanical properties and minimal use of foreign body material.

The Vascular Composite Graft is based on a co-scaffold system using autologous fibrin gel as cell carrier and a macroporous (pore size 1–2 mm) textile PLA structure for mechanical support. Seeded myofibroblasts were isolated from sheep carotid artery. Vascular structures with an inner diameter of 4.5 mm were produced using an injection moulding technique. The tissue-engineered vessels were conditioned using a flow rate of up to 100 ml/min at physiological pulsatile pressure (~120/70/90 mmHg) for 20 days.

Routine histological staining demonstrated adequate growth and morphology of the cells within the fibrin gel surrounding the PLA fibres. Synthesis of types I and III collagen, as well as elastin, was also detected. Furthermore, α SMA expression was evident in the myofibroblasts of the vascular graft. The burst strength of the graft was determined to be >400 mm Hg, underscoring the mechanical stability of the developing tissue.

The Vascular Composite Graft, based on a co-scaffold of a PLA mesh and fibrin gel as a cell carrier, has a mechanically stable structure with remarkable tissue development, and is a potentially implantable structure for the replacement or bypass of diseased or occluded vessels.

(409) Tissue Engineering of the Anterior Cruciate Ligament Using Degradable Phosphate-Based Glass Fibres

Rathbone S., Healy D., Cartmell S.

Institute of Science and Technology in Medicine, Keele University, Medical Research Unit, Hartshill, Stoke-on-Trent, Staffordshire, United Kingdom
Giletech Ltd., 12 North Harbour Estate, Ayr, Scotland KA8 8BN, United Kingdom

Anterior cruciate ligament (ACL) injuries are increasing each year. Approximately 250,000 people are diagnosed with ruptures annually in the USA, and of these approximately 150,000 patients need to undergo surgical treatment.

It is evident that the current surgical treatments for ACL injuries have limitations and do not give completely satisfactory long-term results, therefore tissue engineering is being considered as a possible alternative approach to repair or replace a damaged ACL. This study involves investigating degradable phosphate-based glass fibres as a potentially suitable material for use as a cell scaffold for tissue engineering. The characteristic qualities of this glass makes

it soluble in an aqueous environment, and its dissolution rate is controlled by its composition allowing it to degrade at a rate suiting its application. Primary human fibroblasts derived from the synovial tissue of a patient with an ACL injury have been seeded onto a range of compositions of phosphate-based glass fibres (manufactured by Gilech Ltd., Ayr, Scotland).

Images taken using scanning electron microscopy (SEM) show the fibroblasts attaching to the glass fibres over 4 hours–1 week time points, and secretion of collagenous fibres. Live/dead fluorescent staining has shown cell viability. Cell number has been quantified using a PicoGreen assay indicating that between 16–20% of cells are attaching to the glass fibre samples. We are currently improving cell seeding regimes to increase this cell attachment figure. These initial studies are indicating great potential for these degradable, biocompatible fibres to be used as a cell guiding construct for ACL tissue engineering.

(410) Tissue Engineering Osteochondral Composites Using Co-cultured, Bi-differentiated Adult Mesenchymal Stem Cells

Palmer S.B.^{1,2}, Yang X.², Genever P.¹

¹Biomedical Tissue Research Lab, Department of Biology, University of York, United Kingdom

²Skeletal Tissue Engineering Lab, Department of Oral Biology, Leeds Dental Institute, University of Leeds, United Kingdom

It is hypothesised that molecular signalling interactions between osteoblasts and chondrocytes are important in regenerating a functional osteochondral interface. However, engineering complex skeletal tissues that represent both bone and cartilage is challenging. Mesenchymal stem cells (MSCs) derived from bone marrow can differentiate into osteogenic and chondrogenic lineages. Consequently, MSCs are often the cells of choice for tissue engineered replacement structures for orthopaedic surgery. The primary challenge is to provide appropriate biochemical, physical and mechanical conditions that allow distinct chondrogenic and osteogenic phenotypes to be maintained within the same construct. We have developed a system that allows us to micro-engineer a 3D bone/cartilage construct *in vitro*. In the present work, MSCs were grown as 3D “spheroids” and directed into osteogenic and chondrogenic lineages. Osteo- and chondro-spheroids were subsequently fused to form 3D bi-differentiated structures. The organisation and differentiation status were monitored over a period of time *in vitro*, using histology, RT-PCR, fluorescence microscopy and scanning electron microscopy. The effects of co-culture on the phenotype and gene expression of MSC directed chondrocytes and osteoblasts was also investigated. These results indicated that osteogenic/chondrogenic interactions appear to be important in regulating cell differentiation and maintaining phenotype while the induced osteoblast and chondrocyte MSC spheroids have phenotypic stability for at least six days in culture and establish a clear osteochondral interface. This may provide a useful 3D model for investigation of signaling pathways between bone and cartilage in a biomimetic microenvironment that could lead to novel complex tissues engineering.

(411) Effects of Electromagnetic Fields on Chondrocytes: an *In Vitro* Study

*Kozaci L.D.¹, Bilgin M.D.², Ek R.O.³, Kafienah W.⁴

¹Adnan Menderes University, Faculty of Medicine, Dept. Biochemistry, Aydin 09100, Turkey

²Adnan Menderes University, Faculty of Medicine, Dept. Biophysics, Aydin 09100, Turkey

³Adnan Menderes University, Faculty of Medicine, Dept. Physiology, Aydin 09100, Turkey

⁴University of Bristol Academic Rheumatology, Avon Orthopaedic Centre, Southmead Hospital, Bristol BS10 5NB, United Kingdom

Cartilage tissue engineering involving the patient's own chondrocytes or stem cells, expanding and differentiating them, seeding them onto a biomaterial, and implanting them. However, it is limited by the risk of cell dedifferentiation during proliferation. Techniques with negligible toxic effects are needed to minimize the risk of dedifferentiation and to achieve cell expansion without increasing passage numbers. Chondrocytes are responsive to biophysical stimuli such as electromagnetic field (EMF), ultrasound, and mechanical stresses. EMF plays a regulatory role in cartilage metabolism by increasing the chondrocyte proliferation and synthesis of extracellular matrix components, and reducing the matrix degradation (1, 2).

Aim of this study was to achieve the required chondrocyte numbers without causing any toxicity and dedifferentiation, by reducing the passage numbers in cell cultures by using EMF.

The effects of EMF on chondrocytes, isolated from hyaline cartilage, were investigated in this study. Chondrocytes were isolated from nasal bovine cartilage and cells were cultured with/without growth factors (Insulin-like growth factor or fibroblast growth factor) for 7 days in 24-well plates. Pulsed or continuous EMF was also applied to the plates (30 min/day, 7 days). At the end of culture period, cells were counted. Expression levels of mRNA for type I and II collagens and aggrecan were determined by real-time PCR. In presence of growth factors, EMF exposure induced cell proliferation and matrix protein expression.

EMF may be used as a chondroprotective agent for the treatment of chronic diseases such as osteoarthritis.

References

1. Sollazzo V et al. *Electro Magnetobiol* 1996;15:75–83.
2. Fini M et al. *Journal of Orthopaedic Research* 2005; 23: 899–908.

(412) Tissue Mechanics Can Regulate Gene Expression of Contractile Proteins

Tomasek J.J., Haakma C.J.

Department of Cell Biology, University of Oklahoma-Health Sciences Center, Oklahoma City, OK, 73104, United States of America

One of the critical components for tissue engineering is designing substrates with tissue mechanics suitable to obtain appropriate cell function. We have previously demonstrated that a stiff, adhesive substrate can promote the formation and function of myofibroblasts resulting in tissue contraction and potential anatomical deformation. In this study we have examined the molecular mechanism by which tissue mechanics can regulate the smooth muscle alpha-actin (SMAA) expression, the hallmark of myofibroblast formation and function. We have previously demonstrated that substrate stiffness regulates the intracellular actin filament assembly and focal adhesion formation, as well as extracellular

fibronectin fibril assembly. In addition, the activity of the SMAA promoter in myofibroblasts in granulation tissue is dependent upon the presence of an intronic CARG response element. In this study we present evidence for a molecular model for mechanoregulation of SMAA in myofibroblasts. Myocardin-related transcription factor-A (MRTF-A) can partner with serum response factor (SRF) to active CARG elements in the smooth muscle alpha-actin promoter. G-actin will bind MRTF-A sequestering it in the cytoplasm thereby reducing promoter activity of SMAA. We demonstrate that in response to a stiff, adhesive substratum G-actin is assembled into actin filaments resulting in the release of MRTF-A and its movement into the nucleus, with SRF, to facilitate SMAA promoter activity and thereby enhance myofibroblast differentiation. This model would provide a molecular mechanism whereby a stiff, adhesive substrate can result in mechanoregulated SMAA expression promoting and stabilizing myofibroblast formation and function.

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(413) Tissue Reactions Around the Tissue-Engineered Cartilage-Based on Biopolymers

Fujihara Y., Ogasawara T., Asawa Y., Fujihara H., Takato T., Hoshi K.

The University of Tokyo, Department Cartilage & Bone Regeneration (Fujisoft), Bunkyo-ku, Tokyo, 113-0033, Japan

The University of Tokyo, Department Oral & Maxillofacial Surgery, Bunkyo-ku, Tokyo, 113-0033, Japan

The tissue reactions to the artificial biodegradable polymers in engineered-tissues are regarded to result in deformation or malformation of tissue-engineered constructs *in vivo*. However, full details of such unfavorable reactions still remain unclear. For that, we investigated the early-phase reactions in and around the tissue-engineered cartilage consisted of chondrocytes and poly-L-lactic acid (PLLA) scaffold. The PLLA scaffolds (5×5×3 mm) with or without human auricular chondrocytes (10⁷ cells) were subcutaneously transplanted in nude mice, and time-course changes of augmentation of neovascularization and activation of macrophages, which can be assessed even in the nude mice, were evaluated by means of biochemistry and histochemistry. As a result, tissue hemoglobin level and IL-1β protein content in PLLA alone (PLLA group) were significantly higher than that in the PLLA with chondrocytes (cells/PLLA group) over the observation period (8 weeks), suggesting that the vascular hyperpermeability and activation of macrophage system, which were triggered by PLLA, were suppressed in cells/PLLA group. Immunohistochemical evaluations of F4/80 in PLLA group showed the accumulation of macrophages around PLLA at 2 weeks, while some of them fused to form multinucleated giant cells by 8 weeks. Otherwise, macrophage localization in cells/PLLA group was sparse at 2 weeks and gradually restricted to non-cartilage area thereafter. The macrophages could not be seen around the PLLA that was directly surrounded by maturely regenerated cartilage. These findings indicated that the intensive immunoreactions inevitably occur in and around the artificial biopolymers alone, but that the maturation of tissue-engineered cartilage possibly suppresses such unfavorable reactions.

(414) Towards Auricular Reconstruction Utilizing Composite Constructs Made from Customized Polycaprolactone-Based Scaffolds in Combination with Hydrogels

Baumer J.¹, Eyrych D.^{1,2}, Skodacek D.², Staudenmaier R.³, Wenzel M.³, Welter J.⁴, Kadege G.⁴, Goepferich A.¹, Maier G.⁵, Wiese H.⁵, Blunk T.¹

¹Department of Pharmaceutical Technology, University of Regensburg, Germany

²Department of Otolaryngology, University Hospital of Regensburg, Germany

³Department of ENT, Head and Neck Surgery, Hospital rechts der Isar, Technical University of Munich, Munich, Germany

⁴KL-Technik GmbH, Kraling/Munich, Germany

⁵polyMaterials AG, Kaufbeuren, Germany

Engineering cartilaginous tissue in a complex shape such as needed for auricular reconstruction has been a challenge for years. Particularly if aimed at such complex constructs, employing either hydrogel or solid polymeric scaffold alone as cell carrier prevalently renders unsatisfying results.

Therefore, in the first part of the study, primary bovine chondrocytes were suspended in a long-term stable fibrin gel [1] which was subsequently injected into polycaprolactone-based polyurethane scaffolds (discs: 5 mm diameter, 2 mm thick). After 4 weeks *in vitro*, these composite constructs displayed engineered cartilage with large amounts of extracellular matrix (ECM) components, i.e., glycosaminoglycans and collagen, that were homogeneously distributed throughout the scaffold. In contrast, seeding cells directly onto the solid polyurethane scaffold resulted in lower ECM content and inhomogeneous ECM distribution. Composite constructs implanted into the back of nude mice exhibited, after 1, 3, and 6 months *in vivo*, adequate formation of cartilage tissue with evenly distributed ECM components. Again, in contrast, scaffolds seeded with cells alone showed distinctly inferior tissue development over the whole course of the experiment.

The second part of the study aims at the development of cartilaginous tissue in the shape of a human ear utilizing the established concept of the composite constructs. Customized polycaprolactone-based scaffolds were produced utilizing an ear-shaped silicone mold fabricated by rapid prototyping techniques. Interconnective pore structure and even distribution of injected fibrin gel was demonstrated. *In vitro* development of cartilaginous tissue within the ear-shaped composite constructs is currently under investigation.

Reference

1. Eyrych D et al., *Biomaterials* (2007) 28, 55–65.

(415) Towards Development of Antibody Targeted Liposomes for Gene Delivery

Kulkarni M., Greiser U., Pandit A.

National Centre for Biomedical Engineering and Science, Regenerative Medicine Institute, National University of Ireland, Galway, Ireland

Liposomal systems have many advantages like less immunogenicity, relative ease of large scale fabrication, and unlimited amount of DNA that can be delivered; their low transfection effi-

ciency and lack of specificity are major drawbacks. It was hypothesized that by conjugating Fab fragment of an antibody to the liposomes, the transfection efficiency can be increased and targeted and the absence of Fc fragments will make the system less immunogenic. Antibody targeted liposomes were prepared by conjugation of Fab fragments of anti GM3 IgG antibody to the liposomes via a linker molecule [1]. Briefly, anti GM3 IgG antibodies were first cleaved. The F(ab')₂ thus obtained were then reduced by DTT and conjugated to Maleimide-PEG-PE which were then inserted in the liposomes [2]. The transfection efficiency of these antibody targeted liposomes was tested on B16 F0, a GM3 antigen expressing mouse skin melanoma cell line [3], using a marker plasmid, pEGFP as against liposomes (*n* = 3). The results were evaluated using confocal microscopy and flow cytometry. The antibody targeted liposomes showed significant increase in transfection efficiency in comparison to that of liposomes. Thus, antibody targeted liposomal system can be used for gene delivery. Also, in future studies, this system will be used for tethering the liposomes to a biodegradable scaffold for sustained release of the gene.

References

1. H.A.H. Rongen et al. (1997) *J. Immunol Methods* 204, 2, 105–133.
2. S. Shahinian et al. (2004) *Methods Enzymol* 387, 3–15.
3. M. R. Gabri et al. (2002) *J Cancer Res Clin Oncol* 128, 12, 669–77.

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(416) Type V Collagen Enhances Matrix Contraction by Human Shoulder-Derived Fibroblasts Seeded in Three-Dimensional Collagen Gels

Bains M., Lambert S., Mudera V.

Tissue Repair and Engineering Centre, IOMS, UCL, RNOH, Brockley Hill, Stanmore, HA7 4LP, United Kingdom

Introduction: Extracellular matrix is composed of many components that play a crucial role in modulating cellular activity. Connective tissue contains several types of collagen with concentrations varying in different tissues. We investigated the effect on cellular contraction of ECM using 3-D gels composed of collagen I alone or in combination with 10% collagen V by human shoulder fibroblasts.

Methods: Primary explant cultures of fibroblasts from shoulder capsule and shoulder ligament were obtained using standard tissue culture techniques. Fibroblasts were seeded in 3-D collagen constructs consisting of (i) 100 % Collagen Type I and (ii) 90% Type I and 10% Type V. Contraction of the ECM was measured over 24 hours in both free floating gels and force generation quantified using a culture force monitor (CFM). Electron microscopy was performed on the gels after 24 hours to measure collagen fibril diameter.

Results: Contraction of free floating gels was only seen in seeded gels containing both collagen I and V. Greater force generation was measured in the combination collagen type I and V gels as compared to gels containing just collagen I using the CFM.

Conclusion: The enhanced contraction of fibroblasts stimulated by collagen V may be mediated through integrins (1) or a change in collagen fibril diameter (2). We have shown that collagen V with collagen I may play an important role in connective tissue contraction with changes that may occur during wound healing and tissue regeneration.

References

1. Berendsen et al. 2006 *Matrix Biol* 25(8): 515–522.
2. Spiess et al. 2007 *Microsc Res Tech* 70(1): 18–25.

(417) Uniaxial Stretch Increases Collagen Synthesis in Fibrin-Based Engineered Tendons

Baar K.

Division of Molecular Physiology, University of Dundee, James Black Centre, Dow Street, Dundee, DD1 5EH, United Kingdom

Tendons are composed of a dense array of parallel collagen fibres that function to connect muscle to bone and permit movement. The aim of the current study was to engineer a 3-dimensional (3D) tendon and study its response to mechanical loading. To achieve this aim, cells from the Achilles tendon of mature rats were isolated and engineered into a 3D structure using fibrin gel casting. During the formation of the constructs, the cells contracted around an oval mold and began to produce their own extracellular matrix (ECM). After ten days in culture, the mold was removed and the tendons were loaded into a cyclic strain bioreactor and stretched at 0.1Hz using strains of 110% of casting length. Seven days of strain resulted in a 2.3-fold increase in hydroxyproline concentration, an estimate of total collagen, within the engineered tendons. The immediate effects of stretch were then studied by stretching the constructs for 5, 15, or 30 minutes. Acute strain resulted in increased phosphorylation of a number of signalling proteins including ERK 1/2 (2.14-fold), p38 (2.58-fold), and S6K1 (2.79-fold). In contrast, two others, PKB and focal adhesion kinase (FAK), were unchanged. The finding that FAK was not activated by stretch in 3D conflicts with previous findings in 2D cell culture and may suggest that the 3D system better reflects what occurs *in vivo*. We conclude that tendon cells, embedded in a fibrin gel, are mechanoresponsive and will provide a promising model for studying the effects of loading *in vitro*.

(418) Up-regulation of Bone Morphogenetic Protein Receptor (BMPR) Genes by Orthopaedic and Dental Implant Materials

Singhatanadgit W., Salih V., Olsen I.

BTE Division, UCL Eastman Dental Institute, 256 Gray's Inn Road, London WC1X 8LD, United Kingdom

The bone morphogenetic proteins (BMP) are a family of TGF β -related factors that have a potent effect on bone cell growth and differentiation *in vitro* and on bone formation *in vivo*. They elicit their osteogenic responses via signal transduction mediated by 3 main transmembrane receptors (BMPR-IA, -IB and -II). Numerous studies indicate that implant materials can have a significant impact on bone repair and regeneration, although it is not yet known whether this involves changes in BMPR expression. We have

therefore examined whether implant materials could elicit their beneficial effects by up-regulating BMPR mRNA, thereby enhancing BMP-induced osteogenesis.

Alveolar bone (AB) cells were cultured on stainless steel, titanium, phosphate glass and hydroxyapatite discs for 24 h and RNA isolated using the RNeasy[®] Mini Kit. Semi-quantitative RT-PCR was carried out using the housekeeping gene GAPDH as an internal standard to assess the quality of the extracted RNA and as a baseline for calculating the relative level of BMPR-IA, -IB and -II transcripts.

Gene expressions of the BMPR were markedly up-regulated particularly in cells grown on modified materials when compared to those cells grown on unmodified materials. Expression of BMPR-IA, -IB and -II transcripts was differentially modulated by implant materials which have different chemical and topographical characteristics. In particular, modified hydroxyapatite, roughened titanium and iron-containing phosphate glasses up-regulated the gene expression of all 3 receptors.

This suggests that novel implant materials could be developed to significantly enhance the up-regulation of BMPR expression and thus optimize the osteogenic response of bone cells.

(419) Use of a Rabbit Cornea Model for the Development of a Cell Transfer System for Limbal Epithelial Cells

Deshpande P., Bullett N., Notara M., Daniels J.T., Haddow D. and MacNeil S.

UCL, Institute of Ophthalmology, 11-43 Bath Street, London, EC1V 9EL, United Kingdom

CellTran Ltd, Innovation Centre, 217 Portobello, Sheffield S1 4DP, United Kingdom

University of Sheffield, Department of Engineering Materials, Kroto Research Institute, North Campus, Broad Lane, Sheffield S3 7HQ, United Kingdom

This work involves the development of a contact lens system for transferring laboratory expanded limbal epithelial cells for treatment of the cornea. The approach taken is to use a chemically defined engineered surface using plasma polymerisation technology to develop a coating which supports both the initial attachment of epithelial cells and their subsequent transfer onto the denuded cornea. To assist in this development we have established a rabbit organ culture model to examine transfer of cells onto the cornea. First we examined the culture of a human corneal epithelial cell line and primary rabbit limbal epithelial cells on a range of plasma coatings. Acrylic acid, allyl amine and allyl alcohol surfaces were synthesised at different power and flow rates. From these, the surface which best supported epithelial cell culture (both human and rabbit cells) was identified. Cells were then cultured on contact lenses coated with this surface. Rabbit corneal organ cultures with the intact epithelium were then denuded of epithelial cells. Lenses with cells were placed onto the cornea and kept in place for 4 days. Transfer of cells from lenses was examined by pre-staining cells with CellTracker[™] Red CMTPX and also by subsequent staining of cells on the cornea with DAPI and phalloidin FITC. The results showed that the surface that best supported the epithelial cell culture was acrylic acid. Preliminary results using this model show that the primary rabbit limbal epithelial cells and the human cell line will transfer from the lens onto the cornea.

(420) Use of Aligned Polymer Microfibres for Peripheral Nerve Repair

Murray-Dunning C., Sane N., McKean R., MacNeil S., Ryan A., Haycock J.W.

Departments of Engineering Materials & Chemistry, University of Sheffield, United Kingdom

Nerve guidance conduits (NGC) have considerable potential for repairing peripheral nerve gap injuries caused by trauma, with basic entubulation designs encouraging limited reinnervation of nerve fibres. Following transection injury, Schwann cells are essential for repair as they proliferate rapidly, clear debris and secrete growth factors. We designed a closed loop bioreactor that enables us to seed Schwann cells remotely into an experimental NGC that consists of uniaxially aligned polymer microfibres. RN22 cells were introduced in wet spun aligned viscose rayon fibres (10–20 µm diameter) varying in length from 10–80 mm, contained within 1.2 mm diameter silicone tubes. Cells were grown under static conditions (without flow) and also under static conditions for 2–4 hours followed by continuous flow (0.5–5.0 ml/min) for 24–72 hours. MTT data showed that whilst static culture resulted in constructs with poor viability, cell viability was considerably improved when given an initial 4 hour adhesion time followed by a 0.5 ml/min flow rate. We also investigated seeding RN22 cells into aligned electrospun poly-L-lactic acid (PLLA) fibres (5 µm diameter; 10 mm long) for 48 hours. RN22 cells were stained with live/dead fluorophores (Syto-9 and propidium iodide) and analysed by taking confocal z-stacks. Images revealed a high degree of uniaxial alignment and 95% live cell viability. Cells were also investigated for myelin expression and stained positive on aligned PLLA fibres. In conclusion, the following NGC approach is readily adaptable for autologous or stem cell methods for clinical nerve repair, but also suitable for designing a cell-free device.

(421) Use of Plastic Compressed Collagen Sheet Scaffolds to Engineer a Model of Fascia Tissue

Kureshi A.¹, Hyder Z.¹, Afoke A.², Brown R.A.¹

¹University College London, Tissue Repair and Engineering Centre, Institute of Orthopaedics & Musculoskeletal Science, Stanmore Campus, London HA7 4LP, United Kingdom

²University of Westminster, Department of Information Systems & Computing, Cavendish Campus, London W1W 6UW, United Kingdom

Native collagen gels are important biomimetic scaffolds for tissue engineering but suffer from low mechanical strength and poor structural control. Plastic compression (PC) is a rapid process that removes fluid from hyper-hydrated gels to produce controlled density, strong tissue-like scaffolds rapidly. This study aims to identify the mechanical creep properties of such scaffolds as a precursor to understanding cell-mediated creep of collagen constructs and design an engineered model of fascia tissue. An *in vitro* culture test rig has been developed in which a constant mechanical load is applied over extended periods under culture conditions, with the measurement of real time extension. Static loads were applied to PC collagen sheets (mean thickness was 94 µm ± 13.2) corresponding to 20%, 50% and 70% of the predetermined mean

break stress which was 0.3 MPa ± 0.11. The gradient of the secondary phase of the creep curve (which represents the creep rate) for each specimen was measured. Creep strain rates for 20% (applied stress 0.06 MPa), 50% (0.14 MPa) and 70% (0.2 MPa) of ultimate break stress were measured at: 0.02 %, 0.54% and 3.5% per minute respectively. Hence creep rate increased by 27 fold and 6.5 fold respectively when loading increased between 20% and 50% and between 50% and 70% of break stress. This provides further key understanding of the material properties of PC collagen constructs and represents the basis of a model of engineered fascia tissue.

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(422) Use of Tissue-Engineered Skin to Enhance Understanding of Skin Graft Contraction

Harrison C.A., Thornton D.J., MacNeil S.

Tissue Engineering Group, Department of Engineering Materials and Division of Biomedical Sciences and Medicine, Kroto Research Institute, University of Sheffield North Campus, Broad Lane, Sheffield, S3 7HQ, United Kingdom

Skin graft contraction is an intractable problem. Current treatments focus on mechanical opposition of contractile forces using splints and compression of grafted skin by pressure garments. Despite 50 years of research in this area, treatment and prevention have progressed very little.

The MacNeil group have 10 years experience of developing a tissue-engineered model of human skin based on sterilised human dermis, seeded with expanded human keratinocytes and fibroblasts (1). This contracts by 60% over 28 days culture *in vitro* (2), with the contraction being predominantly due to the keratinocyte rather than the fibroblast. Agents known to enhance keratinocyte differentiation e.g. Vitamin C, increase contraction, whereas keeping the keratinocyte in an undifferentiated form e.g. by lowering extracellular calcium, reduces contraction. Epidermal removal after 10 days culture results in relaxation of the dermis to its original surface area. However, by 30 days, contraction becomes irreversible and the dermis appears to be maintained in its contracted state through collagen crosslinking. Application of mechanical restraint for 8 days reduces both the rate and extent of subsequent contraction. Translation of these approaches into the clinic, by earlier use of splinting, and calcium-chelating or crosslink inhibitor-impregnated dressings may prove effective in combating graft contraction.

References

1. Ghosh M et al. (1997). A comparison of methodologies for the preparation of human epidermal-dermal composites. *Ann Plast Surg* 39:390–404.
2. Harrison CA et al. (2007). Use of an *in vitro* model of tissue-engineered skin to investigate the mechanism of skin graft contraction. *Tissue Eng.* In Press.

(423) Vascularised Cardiac Tissue Engineering *In Vivo* from Minced Rat Cardiac Muscle

Morritt A.N.^{1,2}, Dilley R.J.¹, Bortolotto S.K.¹, Han X.L.¹, Morrison W.A.^{1,2}

¹The Bernard O'Brien Institute of Microsurgery, Melbourne, Australia

²The Department of Surgery, St Vincent's Hospital, Melbourne, Australia

Introduction: Cardiac tissue engineering strategies to date have generally utilised cultured cardiomyocytes, however there is evidence that cell culture may be deleterious for tissue engineering outcomes, introducing foreign serum constituents into cell and matrix components or inducing autoantigen presentation resulting in immunorejection once implanted. We show here that vascularised spontaneously beating cardiac tissue can be generated from neonatal rat ventricle tissue.

Methods: Minced neonatal or adult rat ventricle in Matrigel[®] was implanted with an arteriovenous blood vessel loop into a tissue engineering chamber sited subcutaneously in an adult nude rat. Chambers were harvested 2–10 weeks after insertion and underwent histological analysis.

Results: In the minced neonatal cardiac muscle group, all constructs harvested at 6–10 weeks contained spontaneously contractile tissue. Angiogenesis and lymphangiogenesis were evident throughout the tissue construct. Immunostaining for desmin, α -sarcomeric actin and troponin, showed that cardiomyocytes were present in discrete clusters at all time points. Cardiomyocytes stained positive for connexin-43 and increased in size, and some were positive for the proliferation markers Ki67 and PCNA. In the minced adult heart muscle group, no spontaneous contractile activity was seen.

Conclusion: Minced neonatal rat cardiac muscle survived, grew and formed vascularised spontaneously beating tissue in an *in vivo* vascularised tissue engineering chamber. In contrast, adult rat cardiac muscle did not form spontaneously beating tissue.

(424) VascuPlug (STREP): Biocompatibility Testing and Surface Modifications

Welle A., Missirlis Y., Michanetzi G.

Institute for Biological Interfaces, Forschungszentrum Karlsruhe, PO Box 3640, 76012, Karlsruhe, Germany

The STREP "VascuPlug" is focused on the interdisciplinary development of a vascularized implantable engineered tissue construct. An overview will be presented separately. Main parts of the construct are the porous biodegradable scaffold with predefined capillaries and a drug release system based on nanoparticles.

Several polymers for scaffold production like commercial Poly(L-lactide-co-glycolide) (PLGA), Poly(dioxanone) (PDX), in-house synthesized Poly(ϵ -caprolactone-co-glycolide) and others together with several non-loaded drug delivery particles based on Chitosan/Hyaluronic acid and PLGA/Poloxamer were ranked according to their cyto- and haemocompatibility. Therefore, agar-overlay experiments, cell adhesion tests, mitochondria activity test (XTT), platelet adhesion tests, aPTT, and complement activation tests were performed on native and sterilized samples. Our results indicate that most Poly(ϵ -caprolactone)-based materials are not suitable for the envisaged application due to an insufficient cytocompatibility, whereas PLGA and PDX exhibited no cytotoxicity. Upon inoculation with C3a hepatoma cells on both materials an

initial cell loss was observed. Plasma treatments did not improve the cell adhesion whereas scaffold conditioning by collagen adsorption stimulated the adhesion and proliferation of C3a cells and minimized the cell loss. Hereupon, PLGA was subjected to different foaming technologies (thermal induced phase separation, particle leaching) and the obtained 3D scaffolds underwent further tissue compatibility studies. Porous PLGA cylinders of 10 mm diameter and 3 mm thickness were used for cell culture with constant medium perfusion for 10–20 days. Cells remained viable and colonized the scaffolds.

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(425) Vasoactive Drugs Reduce Smooth Muscle Cell-Mediated Contraction of Fibrin Gel in Culture: Implications for Fibrin-Based Heart Valve Tissue Engineering

Diamantouros S.¹, Flanagan T.C.¹, Sachweh J.S.², Schmitz-Rode T.¹, Jockenhoevel S.¹

¹Department of Applied Medical Engineering, Helmholtz Institute for Biomedical Engineering, RWTH Aachen, Pauwelsstrasse 20, 52074 Aachen, Germany

²Department of Paediatric Cardiac Surgery, University Hospital, Pauwelsstrasse 30, 52074 Aachen, Germany

A limitation of the use of fibrin gel as a tissue-engineered heart valve scaffold is valve insufficiency caused by cell-mediated fibrin gel shrinkage. The current study aims to develop and utilise a fibrin gel contraction assay to evaluate the hypothesis that vasoactive drugs can modulate cell-mediated contraction of fibrin gels.

Fibrin gels containing ovine carotid artery-derived cells (2×10^6 cells/ml) were moulded in 12-well plates. Gels were released from the culture plastic and culture medium was supplemented with one of five drugs (a calcium antagonist, verapamil or diltiazem; beta-receptor agonist, salbutamol or bambuterol; or the alpha-receptor antagonist, phentolamine) at concentrations ranging from 10^{-7} M to 10^{-4} M. Control groups consisted of gels with or without cells cultured in standard medium. Fibrin gel contraction and collagen production over 12 days was quantified, while the cell phenotype and tissue development were analysed using immunohistochemistry.

The panel of drugs was shown to reduce fibrin gel contraction in a dose-dependent manner, with the maximum effect induced by 10^{-4} M phentolamine. Collagen synthesis within the gels was not reduced by any of the drugs. Immunohistochemical analysis of control and phentolamine-supplemented samples demonstrated a similar fibroblast-like morphology of viable, seeded cells, while alpha SMA expression appeared unaffected by the drug. Synthesis patterns of types I and III collagen were similar in both control and phentolamine-supplemented gels.

Phentolamine acts as a strong inhibitor of smooth muscle cell-mediated fibrin gel shrinkage. The supplementation of phentolamine is a promising, attractive approach to reduce the shrinkage and insufficiency of fibrin-based tissue-engineered heart valves.

(426) VEGF165 Overexpression and Membrane Type 1 Metalloproteinase Regulates Endothelial Cells Attraction and Polarization for Neo-vascularisation in Porcine Full Thickness Wounds

Dickens S., Vermeulen P., Hendrickx B., Van Den Berge S., Vranckx J.J.

Laboratory of Plastic Surgery and Tissue Engineering Research, Department of Plastic & Reconstructive Surgery, KULeuven University Hospital, Katholieke Universiteit Leuven, B-3000, Belgium

The intricate wound repair process involves the interplay of numerous cells and proteins. Using a porcine full thickness wound (FTW) healing model, we hypothesized that *ex vivo* gene transfer of VEGF-transfected basal keratinocyte cell suspensions may generate cross talk and induce matrix maturation and angiogenesis. To regulate VEGF165 overexpression and its impact on healing, we introduced a tetracycline-inducible gene switch in the expression plasmid. Secondly, we propose that metalloproteinases (MMP) determine the architecture of the extracellular matrix (ECM) by digesting basement membrane and ECM proteins. Specifically, membrane type 1 MMP (MT1-MMP), expressed by endothelial cells and basal keratinocytes, is known to be involved in the polarization of endothelial cells to form tubules during capillary sprouting.

Autologous basal keratinocytes were cultivated from the porcine donor and transfected by liposomes. FTW were treated with VEGF-transfected keratinocytes (with or without 1 µg/ml tetracycline) versus controls. Wound fluids were collected daily and examined by ELISA. Biopsies were evaluated by immunostaining for fibronectin, CD31, CD144, lectin BS-1 and MT1-MMP.

We obtained upregulated VEGF165 levels by adding tetracycline to the wound environment ($p < 0.01$) and enhanced fibronectin deposition in the ECM ($p < 0.01$) as compared to controls. In the ECM enriched matrix, we also found strong endothelial cell staining and tubuli formation ($p < 0.01$) in wounds treated with VEGF-expressing keratinocytes and elevated levels of MT1-MMP ($p < 0.05$).

In conclusion, MT1-MMP, a vital mediator of angiogenesis, is induced by VEGF165, is present in the fibronectin-rich proangiogenic matrix and represents a potential target for vasculogenic induced tissue repair therapies.

(427) Viability and Antitrombogenic Activity of Endothelial Cells used for Tissue Engineering

Alaminos M., Garcia-Honduvilla N., Rodriguez-Morata A., Gomez-Gil V., Garcia J.M., Pascual G., Bujan J., Campos A.

University of Granada (Spain), University of Alcala (Spain) and Virgen de la Victoria University Hospital, Malaga, Spain

Introduction: Evaluation of cell viability (1) and antitrombogenic activity of endothelial cells is a key subject in vascular tissue engineering research. In this work we have determined prostacyclin levels and quantitative ionic profile of cultured umbilical cord vein endothelial cells (HUVEC) belonging to different subcultures.

Materials and Methods: Primary HUVEC cultures were grown in M-199 medium. The initial endothelial phenotype was characterized using anti-Von Willebrand and anti-CD34 antibodies. Cell cycle of the cultures was evaluated by flow cytometry. Proliferation (PCNA) and apoptosis (TUNEL method) of the cultures were also evaluated. PECAM-1 expression was determined by flow cytometry and immunocytochemistry. Cells were subcultured to the third passage. To determine prostacyclin levels, RIA was used, whereas the intracellular ionic content was quantified by X-Ray microanalysis. We used peak-to-local-background ratio method with reference to salt standards.

Results and Discussion: HUVEC content in primary cultures was 80–90%. HUVEC cultures showed a high proliferation index, with very few cells undergoing apoptosis. Endothelial cells kept their morphological and immunocytochemical features until the 3rd subculture. Nevertheless, a significant reduction of the levels of prostacyclin was observed from the 2nd subculture, whilst our microanalytical results at the third subculture showed the highest K/Na ratio and therefore the highest cell viability. This suggests that cells from second and third subcultures should be, because of its antitrombogenic activity and high cell viability, preferentially used for vascular tissue engineering.

Reference

1. Alaminos et al. (2007). Evaluation of the viability of cultured corneal endothelial cells by quantitative electron probe X-ray microanalysis. *J Cell Physiol.* 211:692–698.

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(428) Viability of PTFE Vascular Grafts Modified by Tissue Engineering

Gomez-Gil V.¹, Pascual G.¹, García-Honduvilla N.², Domínguez B.¹, Escudero C.³, Minguela F.⁴, Bellón J.M.², Bujan J.¹

¹Department of Medical Specialities and ²Department of Surgery, Faculty of Medicine, University of Alcalá, Alcalá de Henares, Madrid, Spain

³Puerta de Hierro Hospital, Service of Experimental Surgery, Madrid, Spain

⁴La Paz Hospital, Service of Vascular Surgery, Madrid, Spain

Objectives: The search for vessel substitutes to replace small/medium calibre arteries is an ongoing concern for vascular surgeons. Current approaches include regeneration therapy techniques designed to find one that resembles the structure and function of native arteries. Vascular constructs (engineered vessels) using autologous endothelial and fibroblastic cells were designed and assessed to inhibit thrombosis and reestenosis after implantation as arterial substitutes.

Methods: Three study groups were established: ePTFE as control vascular grafts ($n = 24$); Vascular constructs: ePTFE seeding with endothelial cells ($n = 12$) and ePTFE seeded with fibroblast cells. Then, 24 h later they were fixed with glycerol and seeded with endothelial cells ($n = 12$). Engineered vessels before implantation were subjected to a custom-designed femoral *ex-vivo* circuit for checking cell retention using ¹²⁵In. All the arterial substitutes were implanted in the carotid of dog and removed after two months. Histological studies were performed.

Results: The *ex-vivo* circuit revealed that the presence of a fibroblast coat induced over double the endothelial cell (EC) retention compared to vessels with only endothelial cells (45.2% vs 20.5%). Engineered vessels showed significant reduction in platelet adhesion after blood flow. In *in vivo* implants, the engineered vessels were more efficient at avoiding occlusion than the ePTFE grafts (25% vs. 66%). The interposition of a fibroblastic matrix induced more endothelialisation than those seeded with EC alone. Intimal hyperplasia response diminished a 25% in the EC substitutes, while

significant differences in apoptotic cells emerged between the EC and the control ePTFE grafts.

Conclusions: Engineered vessels showed improved initial patency over ePTFE grafts combating restenosis, good indicator of the long-term efficiency of the graft.

(429) Viscoelastic Behavior of Individual Collagen Fibrils Tested by AFM

Yang L., Van der Werf K.O., Bennink M.L., Dijkstra P.J., Feijen J.

Polymer Chemistry and Biomaterials, Faculty of Science & Technology and Institute of Biomedical Technology (BMTI), University of Twente, P. O. Box 217, 7500 AE, Enschede, The Netherlands
Biophysical Engineering, Faculty of Science & Technology and MESA+ Institute for Nanotechnology, University of Twente, P. O. Box 217, 7500 AE, Enschede, The Netherlands

The viscoelastic behavior of individual collagen fibrils, a substructure in the hierarchical arrangement of collagen molecules in fibers, is essential for understanding the properties of tissues as well as the use of collagen in tissue engineering. In our previous work we showed that micromechanical bending of collagen fibrils is an excellent method to study the mechanical properties of the fibrils [1]. Recently we studied the viscoelastic behavior of individual collagen type I fibrils isolated from tendon at ambient conditions and in PBS buffer.

Individual collagen fibrils were attached between an AFM cantilever and glass surface. The viscoelastic behavior of the attached collagen fibril was studied by stress-strain and stress-relaxation measurements. Immersed in PBS buffer, the stress strain curve of individual collagen fibrils shows a toe region followed by a linear region. The value of elastic modulus increases from 0.60 GPa at a strain rate 0.1 $\mu\text{m/s}$ to 1.05 GPa at a strain rate 20 $\mu\text{m/s}$. Stress-relaxation during a 5–10 min period was performed at different strain levels. The relaxation process of individual collagen fibrils could be described by the two-term Prony series with relaxation times τ_1 and τ_2 of 1.6 ± 0.6 s and 37 ± 12 s, respectively. The viscous behavior of individual collagen fibril is related to the sliding of microfibrils and molecules in the fibril.

Reference

1. Yang L., van der Werf KO., Koopman HFJM., Subramaniam V., Bennink ML., Dijkstra PJ., Feijen J. (2006). J. Biomed. Mater. Res-A. Published online.

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(430) Vitrification Cryopreservation of Neural Stem Cells

Ma X.H.¹, Lu K.¹, Liu Y.¹, Ge D.¹, Cui Z.F.², Liu T.Q.¹

¹Dalian R & D Center for Stem Cell & Tissue Engineering, Dalian University of Technology, Dalian, 116024, China

²Department of Engineering Science, Oxford University, Parks Road, Oxford OX1 3PJ, United Kingdom

Neural stem cells (NSCs) have the extensive applications in the areas of tissue engineering, regenerative medicine and cell therapy.

The efficient cryopreservation protocols to facilitate their storage and transportation need to be developed. Vitrification is a promising, novel and simple procedure that requires less time and is likely to become more effective than the slow cooling method. Usually, the vitrification with a combination of high concentrations of cryoprotectant agents has very high toxicity and may cause osmotic injuries to cells when the solution is loaded and diluted.

The main objective of this study is to find some more efficient solutes and to determine conditions for vitrification. Cryomicroscopic inspection during the freezing and thawing had been carried out for a series of vitrification solutions. And then, vitrification solution loading procedure including the effect of the operation temperature and exposure time was evaluated. The cell viability after the vitrification of NSC suspension was compared with the results for the conventional slow-cooling method for NSC spheres.

A new CPA including both penetrating and non-penetrating cryoprotectants had been developed for NSC vitrification. Loading at room temperature (20–26°C) within 60 s was prefer to loading at low temperature (0–4°C) within 90 s. The viability of NCSs suspension after vitrification was comparable to that of the neurospheres after slow-cooling. The three neural cell types (neurons, astrocytes and oligodendrocyte) were detected in both the control and vitrified samples after thawing, indicating that the vitrification process did not affect the multipotency of the NSCs.

(431) Vitrification of Corneal Endothelial Cells in a Monolayer

Ma X.H.¹, Fan W.X.¹, Ding L.F.¹, Liu T.Q.¹, Cui Z.F.²

¹Dalian R & D Center for Stem Cell and Tissue Engineering, Dalian University of Technology, Dalian, 116023, China

²Department of Engineering Science, Oxford University, Parks Road, Oxford OX1 3PJ, United Kingdom

The successful preservation of corneas by vitrification has been reported till 2002. Armitage reported recovery of endothelial function after vitrification; but the technique was too time-consuming to be applied in eye banks. To provide the technique for cryopreservation of natural whole corneas and tissue-engineered corneas by vitrification, an *in vitro* model of natural corneal endotheliums, confluent monolayers of bovine corneal endothelial cells (BCECs) in culture was used to examine effect of vitrifying process on the recovery rate of cells. A unique vitrification solution (VS) with a high ratio of nonpenetrating component to the penetrating one was employed, and the loading and removal protocols of CPAs were optimized as well.

The morphological observations by microscopy indicated that the confluent monolayers of BCECs in culture were similar to endotheliums *in situ* both in cell shapes and cell junctions. The VS consisting of 25% (w/w) propane-1,2-diol (PD) and 35% (w/w) trehalose in potassium-rich TES buffered corneal preservation solution (CPTes) was used. An optimal protocol of VS loading and removal was designed by the model predictions with respect to the change of cell volume within ~50–40%, and the intracellular PD concentration above the critical value for vitrification. Vitrification and devitrification transition of the VS were examined using cryomicroscopy and differential scanning calorimetry (DSC). The cryomicroscopic experiment showed the VS can vitrify during freezing and weak devitrification occurred during thawing process, yet it was a slightly different from the results of the DSC test due to the different cooling and heating protocols and the sample weight.

After preservation by vitrification, cell recovery rate based on the OD values measured by cell counting kit-8 (CCK-8), was 61.3%. Further research is needed to improve the preservation effect.

(432) Wound Healing Factors Secreted by Epidermal Keratinocytes and Dermal Fibroblasts in Skin Substitutes

Gibbs S., Spiekstra S.W., Breetveld M., Rustemeyer T., Scheper R.J.

Department of Dermatology, VU University Medical Centre, De Boelelaan 1117, 1081 HV, Amsterdam, The Netherlands

Background: Full skin substitutes, epidermal substitutes and dermal substitutes are currently being used to heal deep burns and chronic ulcers. In this study we investigated which wound healing mediators are released from these constructs and whether keratinocyte–fibroblast interactions are involved.

Methods: Autologous skin substitutes were constructed from human keratinocytes, fibroblasts and acellular donor dermis. Full-thickness skin was used to represent autograft. Secretion of wound healing mediators was investigated by means of protein array, ELISA, neutralizing antibodies and conditioned culture supernatants.

Results: Full skin substitutes and autografts produce high amounts of inflammatory/angiogenic mediators (IL-6, CCL2, CXCL1, CXCL8 and sST2). Epidermal and dermal substitutes produced less of these proteins. Epidermal derived pro-inflammatory cytokines IL-1a and TNF-a were found to synergistically mediate secretion of these wound healing mediators (with exception sST2) from fibroblasts in dermal substitutes. The secretion of pro-inflammatory cytokines (IL-1a, TNF-a), chemokine/mitogen (CCL5) and angiogenic factor (VEGF) by epidermal substitutes and tissue-remodelling factors (TIMP-2, hepatocyte growth factor) by dermal substitutes was not influenced by keratinocyte–fibroblast interactions.

Discussion: Both epidermal derived IL-1a and TNF-a are required to trigger release of dermal derived inflammatory/angiogenic mediators from skin substitutes. The full skin substitute has a greater potential to stimulate wound healing than epidermal or dermal substitutes and is therefore more suitable for chronic wound healing. Dermal substitutes and epidermal substitutes may be more suitable in a two-step protocol for burns wound healing where the wound bed should not become too activated.

(433) High-Throughput Testing of Mechanical Forces Generated in Thin Cell and Tissue Layers

Linder P., Digel I., Temiz Artmann A., Kayser P., Porst D., Artmann G.M.

University of Applied Sciences Aachen, D 52428 Jülich, Ginsterweg 1, Aachen, Germany

HPBioforce[®] is an abbreviation of a new automated high tech device enabling high-throughput tests of cultured cell monolayers,

thin tissue constructs or natural biological membranes for their mechanical properties. It is based on the Celldrum[®] Technology developed in the Laboratory for Cell Biophysics and Bioengineering at the University of Applied Sciences Aachen. In this technology, tissue equivalents exert mechanical forces to a thin silicone membrane. The bending at various stress-conditions, recorded as pressure changes below the membrane vs. strain, is examined resulting in data for the elasticity/stiffness and internal construct tension. A one-Celldrum prototype was developed and used for preliminary investigations to study contracting or relaxing effects of drugs. Besides these “static” cell cultures of connective tissue cells, experiments with self-contracting cardiomyocytes were performed to analyse the effects of epinephrine and beta-blockers. Frequency and forces were recorded simultaneously, giving a new insight into the time course of cell mechanics after drug application. The high-throughput system is integrated inside an incubator and works fully automated. This includes cell culturing, drug application and data sampling. Due to the highly sensitive sensor technology and experiments carried out in the incubator, handling artefacts can be avoided. Finally, big scale drug screening experiments with up to 400 and more samples at a time can be performed simultaneously.

(434) Physical Discontinuities at Body Temperature in Human Red Blood Cells

Artmann G.M.¹, Zerlin K.¹, Digel I.¹, Stadler A.², Zaccai G.³, Temiz Artmann A.¹

¹University of Applied Science, Juelich, Germany

²Institut Laue-Langevin, Grenoble, France

³Laboratory for Neutron Scattering, ETH Zurich & Paul Scherrer Institut, Villigen, Switzerland

In this study, various experiments were carried out with Red Blood Cells (RBC) indicating that some physical properties were different below and above, respectively, body temperature (TB). When aspirating human RBCs, used as simple cell models, into narrow (1.3 µm) pipettes, they undergo a sudden transition from blocking the pipette below to passing the same pipette above TB. If the inner pipette diameter was chosen even narrower (1 µm), RBCs did not pass at all. However, precise cell volume measurements revealed an accelerated inside-to-outside water shift with increasing temperature setting on at TB. Colloid osmotic pressure measurements with whole RBCs in autologous plasma supported this finding. As estimated, this additional bulk water enters the blood plasma compartment in the body leading to a reversible RBC water efflux in an adult person between 37°C and 40°C (fever) of in total ~500 mL. NMR T1-, proton- and light-scattering data showed that hemoglobin molecules inside RBCs become more compact leading to molecular aggregation causing this water efflux. Some of those effects were linked to the species body temperature. This suggests that the physical parameter “body temperature” might be imprinted in hemoglobin’s primary protein structure. There are hints that we might expect discontinuities at body temperature in cells other than RBCs as well.